IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
DOCKET NO. PENT-298-EXT

U.S. Patent No.	:	**5,877,298**
Inventors	:	**Fahim, *et al.***
Assignee	:	**Aventis Pasteur Limited**
Issued	:	**March 2, 1999**
For	:	**ACELLULAR PERTUSSIS VACCINES AND METHODS OF PREPARATION THEREOF**

Mail Stop Hatch-Waxman PTE
Commissioner of Patents
P.O. Box 1450
U.S. Express Mail No. EH243048425US

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Applicant Sanofi Pasteur Limited is the successor-in-interest to Aventis Pasteur Limited (**Exhibit A**) which is the successor-in-interest to Connaught Laboratories Limited (**Exhibit B**). Connaught Laboratories Limited is the assignee of the entire interest in United States Patent No. 5,877,298 by virtue of assignments executed July 5, 1995 and recorded as Reel/Frame numbers 7643/0991 and 7645/0001 in the United States Patent and Trademark Office (USPTO) on October 3, 1995 (**Exhibit C**).

Applicant Sanofi Pasteur Limited submits this Application for Extension of Patent Term under 35 U.S.C. § 156 and provides the following information in accordance with 37 C.F.R. § 1.710, et seq. An application for extension of the term of U.S. Pat. No. 6,696,065 B1, also directed to the PENTACEL® product, is being filed separately. The numbering of the following paragraphs corresponds to the numbering of the requirements for an application set forth in 37 C.F.R. § 1.740.

(1) A complete identification of the approved product as by appropriate chemical and generic name, physical structure, or characteristics.

The approved product is PENTACEL® [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactived Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine] (DTaP-IPV/Hib), a vaccine for intramuscular injection (see Packaging Insert, attached as **Exhibit D**). It consists of Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactived Poliovirus (DTaP-IPV) component and an ActHIB® vaccine component. ActHIB® vaccine (Haemophilus b Conjugate [Tetanus Toxoid Conjugate]), consists of *Haemophilus influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate [PRP]) covalently bound to tetanus toxoid (PRP-T). The DTaP-IPV component is supplied as a sterile liquid used to reconstitute the lyophilized ActHIB® vaccine component to form PENTACEL® vaccine.

PENTACEL® has been approved by the Food and Drug Administration (FDA) for active immunization against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease due to *Haemophilus influenzae* type b in children 6 weeks through 4 years of age (prior to fifth birthday).

Each 0.5 ml dose of PENTACEL® includes the following active ingredients:

diphtheria toxoid	15 Lf
tetanus toxoid	5 Lf
acellular pertussis antigens:	
pertussis toxin (PT) detoxified	20 μg
filamentous haemagglutinin (FHA)	20 μg
pertactin (PRN)	3 μg
fimbriae types 2 and 3 (FIM)	5 μg
inactived polioviruses (IPV):	
Type I (Mahoney)	40 D-antigen units
Type II (MEF-1)	8 D-antigen units
Type III (Saukett)	32 D-antigen units
PRP of *Haemophilus influenzae* type b covalently bound to:	
24 μg of tetanus toxoid (PRP-T)	10 μg

Other ingredients per 0.5 ml dose include 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, polysorbate 80 (approximately 10 ppm by calculation), ≤5 mg residual formaldehyde, <50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative) and <4 pg of neomycin and <4 pg polymyxin B sulfate.

Corynebacterium diphtheriae is grown in modified Mueller's growth medium. After purification by ammonium sulfate fractionation, the diphtheria toxin is detoxified with formaldehyde and diafiltered. *Clostridium tetani* is grown in modified Mueller-Miller casamino acid medium. Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

The acellular pertussis vaccine components are produced from *Bordetella pertussis* cultures grown in Stainer-Scholte medium modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. FIM are extracted and copurified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT detoxified with glutaraldehyde. FHA is treated with formaldehyde and residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate.

IPV consists of poliovirus Type 1, Type II and Type III, which are each grown in separate cultures of MRC-5 cells (a cell line of normal human diploid cells) by the microcarrier method. The cells are grown in CMRL (Connaught Medical Research Laboratories) 1969 medium supplemented with calf serum. For viral growth, the culture medium is replaced by Medium 199, without calf serum. After clarification and filtration, the viral suspensions are concentrated by ultrafiltration and purified by liquid chromatography steps. The monovalent viral suspensions are inactivated with formaldehyde. Monovalent concentrates of each inactivated poliovirus are combined to produce the IPV trivalent poliovirus concentrate.

The adsorbed diphtheria, tetanus and acellular pertussis antigens are combined into an intermediate concentrate. The trivalent poliovirus concentrate is added and the

DTaP-IPV component is diluted to its final concentration. The DTaP-IPV component does not contain a preservative.

PRP, a high molecular weight polymer, is prepared from the *Haemophilus influenzae* type b strain 1482 grown in a semi-synthetic medium. The tetanus toxoid for conjugation to PRP is prepared by ammonium sulfate purification and formalin inactivation of the toxin from cultures of *Clostridium tetani* (Harvard strain) grown in a modified Mueller and Miller medium. The toxoid is filter-sterilized prior to the conjugation process.

The ActHIB® vaccine component does not contain a preservative. Potency of the ActHIB® vaccine component is specified on each lot by limits on the content of PRP polysaccharide and protein per dose and the proportion of polysaccharide and protein that is characterized as high molecular weight conjugate.

PENTACEL® is provided as a liquid DTaP-IPV component and a lyophilized ActHIB® component. Immediately prior to administration, the DTaP-IPV liquid component is injected into the vial containing lyophilized ActHIB® and mixed until a cloudy, uniform suspension results. The 0.5 ml dose is then administered intramuscularly.

(2) A complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred.

The regulatory review occurred under Section 351 of the Public Health Service Act, 42 U.S.C. § 262.

(3) An identification of the date on which the product received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period occurred.

PENTACEL® was first approved by the FDA for commercial marketing on June 20, 2008. A copy of the FDA's approval letter is attached as **Exhibit E.**

(4) An identification of each active ingredient in the product and as to each active ingredient, a statement that it has not been previously approved for commercial marketing or use under the Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, or a statement of when the active ingredient was approved for commercial marketing or use (either alone or in combination with other active ingredients), the use for which it was approved, and the provision of law under which it was approved.

The active ingredients in PENTACEL® are Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed (pertussis toxoid, filamentous haemagglutinin (FHA), pertactin (PRN), fimbriae types 2 and 3), Inactivated Poliovirus, Types I, II and III ("IPV"), and Haemophilus b Conjugate (Tetanus Toxoid Conjugate; "ActHIB®") as a combined vaccine referred to as "DTaP-IPV/Hib Combined" and as described in the attached Packaging Insert (**Exhibit D**). PENTACEL® is the first vaccine shown to simultaneously provide protection against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease due to Haemophilus influenzae type b as a single composition. PENTACEL® was subject to regulatory review separate from that of any of the individual active ingredients. In the clinical trials undertaken as part of the regulatory review process, it was shown that the immunogenicity of each individual active ingredient in PENTACEL® was maintained without compromising the immunogenicity of any of the other individual active ingredients.

The combination vaccine PENTACEL® has not been previously approved for commercial marketing or use under the Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act. Although the individual active ingredients included in PENTACEL® were each previously approved for commercial marketing or use under the Food, Drug, and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act, as further explained below, PENTACEL® is the first approved vaccine comprised of Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed (pertussis toxoid, FHA, PRN, fimbriae types 2 and 3), IPV and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) as a combined vaccine:

- Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed was approved as DAPTACEL® under BLA 103666 on May 14, 2002, albeit the

Acellular Pertussis Adsorbed component of this vaccine, contains less detoxified PT and FHA as that of the Acellular Pertussis Adsorbed of PENTACEL®. More specifically, PENTACEL® contains twice as much detoxified PT and four times as much FHA as DAPTACEL®.

- IPV (MRC-5) was approved under BLA STN: BL 103940 as POLIOVAX® on Nov. 20, 1987.
- Haemophilus b Conjugate was approved under BLA STN: BL 103935 as ActHIB® on March 19, 1992.

The electronic Biologics License Application (eBLA) for DTaP-IPV/Hib was approved on June 20, 2008 for active immunization against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease due to *Haemophilus influenzae* type b in children 6 weeks through 4 years of age (prior to fifth birthday) pursuant to Section 351 of the Public Health Service Act, 42 U.S.C. § 262.

(5) A statement that the application is being submitted within the 60-day period permitted for submission pursuant to 37 C.F.R. §1.720(f) and an identification of the date of the last day on which the application could be submitted.

The product was approved for commercial marketing by the FDA on June 20, 2008. The last day of the 60-day period on which this application could be submitted is August 18, 2008. As this submission is being made before August 18, 2008, it has been timely filed.

(6) A complete identification of the patent for which an extension is being sought by the name of the inventor, the patent number, date of issue, and the date of expiration.

Inventors:	Raafat E.F. Fahim, *et. al.*
U.S. Patent No.:	5,877,298
Date of Issue:	March 2, 1999
Date of Expiration:	March 2, 2016

(7) A copy of the patent for which an extension is being sought, including the entire specification (including claims) and drawings.

A copy of the patent subject to this request is attached as **Exhibit F**.

(8) A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate issued in the patent.

A Certificate of Correction was issued on October 26, 1999, a copy of which is attached as **Exhibit G**. No other certificate of correction, disclaimer, or reexamination certificate has been issued in connection with this patent. A copy of the maintenance fee statement from the USPTO website is attached as **Exhibit H**.

(9) A statement that the patent claims the approved product or a method of using or manufacturing the approved product, and a showing which lists each applicable patent claim and demonstrates the manner in which at least one applicable patent claim reads on the approved product or method of using or manufacturing the approved product.

U.S. Patent No. 5,877,298 claims processes for producing fimbriae types 2 and 3 (fimbrial agglutinogen 2 and 3) antigens from *Bordetella*, which are components of PENTACEL®. Claims 1 to 12 are relevant. These claims are as follows:

1. A process for preparing an agglutinogen preparation comprising fimbrial agglutinogens 2 (Agg 2) and fimbrial agglutinogen 3 (Agg 3) free from agglutinogen 1 from a Bordetella strain, comprising the steps of:

   (a) providing a cell paste of the Bordetella strain;

   (b) selectively extracting fimbrial agglutinogens 2 and 3 from the cell paste by dispersing the cell paste in a buffer comprising about 1M to about 6M urea to produce a first supernatant containing said agglutinogens 2 and 3 and a first residual precipitate;

   (c) separating the first supernatant from the first residual precipitate;

   (d) incubating the first supernatant at a temperature of about 75° C. to about 85° C. and for a time of about 10 minutes to about 60 minutes to produce a clarified supernatant containing fimbrial agglutinogens 2 and

3 and a second precipitate containing non-fimbrial agglutinogen contaminants;

(e) concentrating the clarified supernatant to produce a crude fimbrial agglutinogen solution by precipitating fimbrial agglutinogens 2 and 3 from the clarified supernatant by the addition of a polyethylene glycol to the clarified supernatant, separating the precipitated fimbrial agglutinogen 2 and 3 from the resulting supernatant and solubilizing the separated fimbrial agglutinogens 2 and 3; and

(f) purifying fimbrial agglutinogens 2 and 3 from the crude fimbrial agglutinogen solution to produce the fimbrial agglutinogen preparation comprising fimbrial agglutinogens 2 and 3.

2. The process of claim 1 wherein the temperature is about 80° C.

3. The process of claim 1 wherein the time is about 30 minutes.

4. The process of claim 1 wherein the first supernatant is concentrated prior to the incubation step (d).

5. The process of claim 1 wherein said precipitation is effected by adding polyethylene glycol of molecular weight about 8000 to the clarified supernatant to a concentration of about 3% to about 5 wt. % to effect precipitation of said agglutinogens from the clarified supernatant.

6. The process of claim 5 wherein the concentration of polyethylene glycol is about 4.3 to about 4.7wt%.

7. The process of claim 1 wherein the agglutinogens are purified from the crude fimbrial agglutinogen solution by column chromatography.

8. The process of claim 7 wherein said column chromatography includes SEPHADEX 6B and/or PEI silica column chromatography.

9. The process of claim 7 wherein said purification step includes sterilization of run through from said column chromatography purification to provide a sterile fimbrial agglutinogen preparation.

10. The process of claim 9 wherein said sterile fimbrial agglutinogen preparation is absorbed onto a mineral salt adjuvant.

11. The process of claim 10 wherein said mineral salt adjuvant is alum.

12. The process of claim 1 wherein the Bordetella strain is a strain of *Bordetella pertussis*.

The following is a demonstration of the manner in which claims 1 to 12 read on the approved product:

Claim	Demonstration
1. A process for preparing an agglutinogen preparation comprising fimbrial agglutinogens 2 (Agg 2) and fimbrial agglutinogen 3 (Agg 3) free from agglutinogen 1 from a Bordetella strain, comprising the steps of: (a) providing a cell paste of the Bordetella strain; (b) selectively extracting fimbrial agglutinogens 2 and 3 from the cell paste by dispersing the cell paste in a buffer comprising about 1M to about 6M urea to produce a first supernatant containing said agglutinogens 2 and 3 and a first residual precipitate; (c) separating the first supernatant from the first residual precipitate; (d) incubating the first supernatant at a temperature of about 75° C to about 85° C. and for a time of about 10 minutes to about 60 minutes to produce a clarified supernatant containing fimbrial agglutinogens 2 and 3 and a second precipitate containing non-fimbrial agglutinogen contaminants; (e) concentrating the clarified supernatant to produce a crude fimbrial agglutinogen solution by precipitating fimbrial agglutinogens 2 and 3 from the clarified supernatant by the addition of a polyethylene glycol to the clarified supernatant, separating the precipitated fimbrial agglutinogen 2 and 3 from the resulting supernatant and solubilizing the separated fimbrial agglutinogens 2 and 3; and (f) purifying fimbrial agglutinogens 2 and 3 from the crude fimbrial agglutinogen solution to produce the fimbrial agglutinogen preparation comprising fimbrial agglutinogens 2 and 3.	Each single dose of PENTACEL® for administration to humans includes 5 μg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 1.

Claim	Demonstration
2. The process of claim 1 wherein the temperature is about 80° C.	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 2.
3. The process of claim 1 wherein the time is about 30 minutes.	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 3.
4. The process of claim 1 wherein the first supernatant is concentrated prior to the incubation step (d).	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 4.
5. The process of claim 1 wherein said precipitation is effected by adding polyethylene glycol of molecular weight about 8000 to the clarified supernatant to a concentration of about 3% to about 5 wt. % to effect precipitation of said agglutinogens from the clarified supernatant.	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 5.
6. The process of claim 5 wherein the concentration of polyethylene glycol is about 4.3 to about 4.7wt%.	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 6.
7. The process of claim 1 wherein the agglutinogens are purified from the crude fimbrial agglutinogen solution by column chromatography.	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 7.
8. The process of claim 7 wherein said column chromatography includes SEPHADEX 6B and/or PEI silica column chromatography.	Each single dose of PENTACEL® for administration to humans includes 5 µg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 8.

Claim	Demonstration
9. The process of claim 7 wherein said purification step includes sterilization of run through from said column chromatography purification to provide a sterile fimbrial agglutinogen preparation.	Each single dose of PENTACEL® for administration to humans includes 5 μg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 9.
10. The process of claim 9 wherein said sterile fimbrial agglutinogen preparation is absorbed onto a mineral salt adjuvant.	It is noted that claim 10 incorrectly notes the preparation is absorbed as opposed to adsorbed. The substitution of the letter "d" with the letter "b" is a clerical error which the applicant will seek to have corrected by Certificate of Correction. This clerical error would be immediately apparent to a person skilled in the art to which US Patent 5,877,298 pertains. Each single dose of PENTACEL® for administration to humans includes 5 μg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) adsorbed onto aluminum phosphate prepared as described in claim 10.
11. The process of claim 10 wherein said mineral salt adjuvant is alum.	Each single dose of PENTACEL® for administration to humans includes 5 μg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared as described in claim 11.
12. The process of claim 1 wherein the Bordetella strain is a strain of *Bordetella pertussis*.	Each single dose of PENTACEL® for administration to humans includes 5 μg fimbriae types 2 and 3 (fimbrial agglutinogens types 2 and 3) prepared from *Bordetella pertussis* as described in claim 12.

(10) A statement, beginning on a new page, of the relevant dates and information pursuant to 35 U.S.C. § 156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period as follows:

(i) For a patent that claims a human biological product, the effective date of the investigational new drug (IND) application and the IND number; the date on which a biological license application (BLA) was initially submitted and the BLA number; and the date on which the BLA issued.

The investigational new drug (IND) submission for the investigational use of PENTACEL® was filed with the FDA by Connaught Laboratories Limited (doing business under the business name, Pasteur Merieux Connaught) on July 21, 1999. It was assigned reference number BB-IND 8502 by the FDA and became effective on August 21, 1999.

The electronic biological license application (eBLA) for this product was submitted on July 26, 2005 by Sanofi Pasteur Limited and was assigned reference number STN BL 125145 by the FDA. The FDA approved the eBLA for this product on June 20, 2008 under reference number STN BL 125145.

(11) A brief description beginning on a new page of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities.

- July 21, 1999: The IND submission for the investigational use of PENTACEL® was filed with the FDA by Connaught Laboratories Limited (doing business under the business name, Pasteur Merieux Connaught).

- August 21, 1999: The IND for PENTACEL® (assigned reference number BB-IND 8502) became effective.

- December 29, 1999: The first clinical trial, "494-01, Stage I", was initiated.

- July 26, 2005: eBLA for PENTACEL® was filed with FDA by Sanofi Pasteur Limited and assigned reference number STN BL 125145.

- April 16, 2007: The final clinical trial, "M5A10 Stage I", was completed.

- June 20, 2008: BLA reference number STN BL 125145 for PENTACEL® was issued to Sanofi Pasteur Limited.

- A chart listing the start date and end date of each clinical trial performed in support of the PENTACEL® submission is attached as **Exhibit I**.

(12) A statement beginning on a new page that, in the opinion of the applicant, the patent is eligible for the extension and a statement as to the length of extension claimed, including how the length of extension was determined.

(i) The applicant Sanofi Pasteur Limited is of the opinion that U.S. Patent No. 5,877,298 is eligible for extension under 35 U.S.C. § 156 as it satisfies all of the requirements for extension, as follows:

(a) 35 U.S.C. § 156(a): U.S. Patent No. 5,877,298 claims processes for producing compositions having the characteristics of PENTACEL®.

(b) 35 U.S.C. § 156(a)(1): The term of U.S. Patent No. 5,877,298 has not expired before submission of this application.

(c) 35 U.S.C. § 156(a)(2): The term of U.S. Patent No. 5,877,298 has never been extended pursuant to 35 U.S.C. § 156.

(d) 35 U.S.C. § 156(a)(3): This application is being submitted by the owner of record of the patent in accordance with the requirements of 35 U.S.C. § 156(d).

(e) 35 U.S.C. § 156(a)(4): The approved product PENTACEL® has been subject to a regulatory review period before its commercial marketing or use.

(f) 35 U.S.C. § 156(a)(5)(A): The commercial marketing or use of the approved product PENTACEL® after the regulatory review period is the first permitted commercial marketing or use of the product under the Public Health Service Act, 42 U.S.C. § 262.

(g) 35 U.S.C. § 156(c)(4): No other patent has been extended for the same regulatory review period for any product.

(ii) The length of extension claimed by Applicant Sanofi Pasteur Limited is 5 years and 0 days, until March 2, 2021.

(a) Pursuant to 37 C.F.R. § 1.775(c), the regulatory review period under 35 U.S.C. § 156(g)(1)(B) began on August 21, 1999, and ended on June 20, 2008, which is a total of 8 years and 306 days. This is the sum of the two phases described below:

(1) The "testing phase" under 37 C.F.R. § 1.775(c)(1) began on August 21, 1999, and ended on July 26, 2005, 5 years and 340 days; and

(2) The "application phase" under 37 C.F.R. § 1.775(c)(2) began on July 26, 2005, and ended on June 20, 2008, for a total of 2 years and 331 days.

(b) Pursuant to 37 C.F.R. § 1.775(d), the length of the patent term extension is calculated as follows:

(1) The number of days subtracted from the regulatory review period is computed as the sum of the following:

(i) The number of days in the regulatory review period which were on or before the date on which the patent was issued is 0 years and 0 days; and

(ii) The number of days during which the applicant and its predecessor did not act with due diligence is 0 days; and

(iii) One-half the number of days remaining in the testing phase after subtracting the number of days in the previous two subparagraphs (0 years and 0 days) is 2 years and 353 days.

(2) When the total number of days calculated in (1) (i.e. 2 years and 353 days) is subtracted from the regulatory review period (i.e. 8 years and 306 days), the result is 5 years and 318 days. This total of 5 years and 318 days, when added to the term of the patent

(expiring March 2, 2016), would result in the date January 14, 2022.

(3) Fourteen years, when added to the date of BLA issuance (i.e. June 20, 2008) would result in the date June 20, 2022.

(4) The earlier date of those in paragraphs 2 and 3 above is June 20, 2022.

(5) Five years when added to the date of patent expiration (i.e. March 2, 2016) is March 2, 2021.

(6) The earlier date of those in paragraphs (4) and (5) above is March 2, 2021.

The applicant Sanofi Pasteur Limited is therefore requesting an extension of 5 years and 0 days, until March 2, 2021.

(13) A statement that applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information that is material to the determination of entitlement to the extension sought.

Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information that is material to the determination of entitlement to the extension sought.

(14) The prescribed fee for receiving and acting upon the application for extension.

The undersigned hereby authorizes the Commissioner to deduct the $1,120.00 fee, along with any other fees that may be due with this submission, from Deposit Account No. 50-0244. Alternatively, please charge the credit card shown on the attached PTO-2038.

(15) The name, address, and telephone number of the person to whom inquiries and correspondence relating to the application for patent term extension are to be directed.

Reza Yacoob
Director, Intellectual Property
Sanofi Pasteur Limited
1755 Steeles Ave.
Ontario, Toronto, Canada

Respectfully submitted,

By: Patrick J. Halloran

Date: August 15, 2008

Exhibits

A: Certificate of Amendment of Certificate of Incorporation of Aventis Pasteur Limited showing name change to Sanofi Pasteur Limited

B: Certificate of Amendment of Certificate of Incorporation of Connaught Laboratories Limited showing name change to Aventis Pasteur Limited

C: Assignment of U.S. Pat. No. 5,877,298 from each inventor to Aventis Pasteur Limited

D: Approved Packaging Insert for PENTACEL®

E: FDA approval letter for PENTACEL®

F: U.S. Patent No. 5,877,298

G: Certificate of Correction dated October 26, 1999

H: Copy of Maintenance Fee Statement for U.S. Pat. No. 5,877,298

I: Chart listing the start date and end date of each PENTACEL® clinical trial

Exhibit A

1

Ministry of Consumer and Business Services

Ministère des Services aux consommateurs et aux entreprises

Ontario Corporation Number
Numéro de la société en Ontario

257852

CERTIFICATE
This is to certify that these articles are effective on

CERTIFICAT
Ceci certifie que les présents statuts entrent en vigueur le

DECEMBER 16 DÉCEMBRE, 2004

Director / Directrice
Business Corporations Act / Loi sur les sociétés par actions

Form 3
Business Corporations Act

Formule 3
Loi sur les sociétés par actions

ARTICLES OF AMENDMENT
STATUTS DE MODIFICATION

1. The name of the corporation is: (Set out in BLOCK CAPITAL LETTERS)
Dénomination sociale actuelle de la société (écrire en LETTRES MAJUSCULES SEULEMENT) :

AVENTIS PASTEUR LIMITED/AVENTIS PASTEUR LIMITÉE

2. The name of the corporation is changed to (if applicable): (Set out in BLOCK CAPITAL LETTERS)
Nouvelle dénomination sociale de la société (s'il y a lieu) (écrire en LETTRES MAJUSCULES SEULEMENT) :

SANOFI PASTEUR LIMITED/SANOFI PASTEUR LIMITÉE

3. Date of incorporation/amalgamation:
Date de la constitution ou de la fusion :

1972-06-22
(Year, Month, Day)
(année, mois, jour)

4. **Complete only if there is a change in the number of directors or the minimum / maximum number of directors.**
Il faut remplir cette partie seulement si le nombre d'administrateurs ou si le nombre minimal ou maximal d'administrateurs a changé.

Number of directors is/are: or minimum and maximum number of directors is/are:
Nombre d'administrateurs : ou *nombres minimum et maximum d'administrateurs :*

Number / Nombre	or / ou	minimum and maximum / *minimum et maximum*

5. The articles of the corporation are amended as follows:
Les statuts de la société sont modifiés de la façon suivante :

07119 (03/2003)

6. The amendment has been duly authorized as required by sections 168 and 170 (as applicable) of the *Business Corporations Act.*
La modification a été dûment autorisée conformément aux articles 168 et 170 (selon le cas) de la Loi sur les sociétés par actions.

7. The resolution authorizing the amendment was approved by the shareholders/directors (as applicable) of the corporation on
Les actionnaires ou les administrateurs (selon le cas) de la société ont approuvé la résolution autorisant la modification le

2004,12,16

(Year, Month, Day)
(année, mois, jour)

These articles are signed in duplicate.
Les présents statuts sont signés en double exemplaire.

AVENTIS PASTEUR LIMITED/AVENTIS PASTEUR LIMITÉE

(Name of Corporation) (If the name is to be changed by these articles set out current name)
(Dénomination sociale de la société) (Si l'on demande un changement de nom, indiquer ci-dessus la dénomination sociale actuelle).

By/
Par :

(Signature)
(Signature)

PRESIDENT

(Description of Office)
(Fonction)

EXHIBIT B

For Ministry Use Only
À l'usage exclusif du ministère

Ontario Corporation Number
Numéro de la compagnie en Ontario
257852

1.

Ministry of Consumer and Commercial Relations
Ontario

Ministère de la Consommation et du Commerce

CERTIFICATE
This is to certify that these articles are effective on

CERTIFICAT
Ceci certifie que les présents statuts entrent en vigueur le

DECEMBER 10 DÉCEMBRE, 1999

Director / Directeur
Business Corporations Act / Loi sur les sociétés par actions

Form 3
Business Corporations Act

Formule numéro 3
Loi sur les compagnies

ARTICLES OF AMENDMENT
STATUTS DE MODIFICATION

1. The name of the corporation is: *Dénomination sociale de la société:*

CONNAUGHT LABORATORIES LIMITED

2. The name of the corporation is changed to (if applicable): *Nouvelle dénomination sociale de la société (s'il y a lieu):*

AVENTIS PASTEUR LIMITED / AVENTIS PASTEUR LIMITÉE

3. Date of incorporation/amalgamation: *Date de la constitution ou de la fusion:*

1972 06 22
(Year, Month, Day)
(année, mois, jour)

4. The articles of the corporation are amended as follows: *Les statuts de la société sont modifiés de la façon suivante.*

To change the name of the Corporation to:

AVENTIS PASTEUR LIMITED/
AVENTIS PASTEUR LIMITÉE

5. The amendment has been duly authorized as required by Sections 168 & 170 (as applicable) of the Business Corporations Act.

La modification a été dûment autorisée conformément aux articles 168 et 170 (selon le cas) de la Loi sur les sociétés par actions.

6. The resolution authorizing the amendment was approved by the shareholders/directors (as applicable) of the corporation on

Les actionnaires ou les administrateurs (selon le cas) de la société ont approuvé la résolution autorisant la modification le

1999 12 10

(Year, Month, Day)
(année, mois, jour)

These articles are signed in duplicate.

Les présents statuts sont signés en double exemplaire.

CONNAUGHT LABORATORIES LIMITED

(Name of Corporation)
(Dénomination sociale de la société)

By:/*Par:* _____

(Signature) (Description of Office)
(Signature) *(Fonction)*

J. Mark Lievonen, President

EXHIBIT C

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

1038-422

FEBRUARY 10, 1996

PTAS

MICHAEL I. STEWART
SIM & MCBURNEY
SUITE 701, 330 UNIVERSITY AVENUE
TORONTO, ONTARIO CANADA, M5G 1R7

100074[illegible]

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231.

RECORDATION DATE: 10/03/1995
REEL/FRAME: 7643/0991
NUMBER OF PAGES: 5

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
VOSE, JOHN R.
DOC DATE: 07/05/1995

ASSIGNEE:
CONNAUGHT LABORATORIES LIMITED
1755 STEELES AVENUE WEST
WILLOWDALE, ONTARIO, CANADA M2R 3T4

SERIAL NUMBER: 08433646
FILING DATE: 05/04/1995
PATENT NUMBER:
ISSUE DATE:

JERYL MCDOWELL, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

10-06-1995

581 D

100074996

PTO-1595
(Rev. 6-93)

RECORDATION FORM COVER SHEET
PATENTS ONLY

10-3-95

U.S. Dept. of Commerce
Patent and Trademark Office

Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231.

Sir:

Please record the original documents or copy thereof.

1. Name of conveying party: JOHN R. VOSE

Additional Names Attached? __ Yes _X_ No

2. Name and address of receiving party(ies)

Name: CONNAUGHT LABORATORIES LIMITED

Internal Address: _____

Street Address: 1755 Steeles Avenue West

City: Willowdale State: Ontario Zip: M2R 3T4

Additional Names Attached? __ Yes _X_ No

3. Nature of Conveyance:

_X_ Assignment
___ Security Agreement
___ Merger
___ Change of Name
___ Other _____

Execution Date: July 5, 1995

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: _____.

A. Patent Application No.(s) 08/433,646

B. Patent No.(s) _____

Additional numbers attached? __ Yes _X_ No

ASSIGNMENT

WHEREAS, I, JOHN R. VOSE, a citizen of Canada, residing at 54 bis Route de Paris, 69260 Charbonnieres-les-bains, France, have invented certain new and useful improvements in **ACELLULAR PERTUSSIS VACCINES AND METHODS OF PREPARATION THEREOF**, for which invention I executed an application for Letters Patent of the United States of America on July 17, 1995; and

WHEREAS, I verily believe myself to be an original, inventors of the invention set forth in said application for Letters Patent; and

WHEREAS, CONNAUGHT LABORATORIES LIMITED, a company organized and existing under the laws of the Province of Ontario, sometimes referred to as "said assignee" having a place of business at 1755 Steeles Avenue West, Willowdale, Ontario, Canada, M2R 3T4, is desirous of acquiring the entire and exclusive right, title and interest in and to said invention, any and all patent applications which may be filed thereon, and any and all Letters Patent which may be granted or issued thereon in the United States and throughout the world, including any and all divisions, continuations, reissues and extensions of any of the foregoing;

NOW, THEREFORE, in consideration of the sum of One Dollar ($1.00), to me in hand paid by the said CONNAUGHT LABORATORIES LIMITED, and other good and valuable consideration, receipt of which is hereby acknowledged, I, the said JOHN R. VOSE, have sold, assigned, transferred and set over and do hereby sell, assign, transfer and set over unto the said assignee, its successors and assigns, the entire and exclusive right, title and interest in and to said invention, patent applications and Letters Patent which may be granted or issued for said invention in the United States and throughout the world, including all divisions, continuations, reissues and extensions thereof and all international priority rights associated therewith all to be held by me had this assignment not been made, and I hereby authorize and request the Commissioner of Patents and

Trademarks of the United States and the duly constituted authorities of all foreign countries of the world to issue all Letters Patent relating to the foregoing rights to said assignee, its successors and assigns;

AND I further agree that I will promptly, upon request and without further compensation, do all lawful acts including the execution of all necessary documents, and the giving of testimony that in the opinion of the said assignee, its successors and assigns may be necessary or desirable for obtaining, sustaining, or reissuing United States and foreign Letters Patent relating to the foregoing assigned rights, and for perfecting, affirming, recording and maintaining the title of said assignee, its successors and assigns thereto, and that we will generally cooperate to the fullest extent in all matters pertaining to said invention and patents, and said assignee's title thereto.

IN WITNESS WHEREOF, I have hereunto set my hands and seal this day of AUGUST 30th, 1995.

JOHN R. VOSE

On this day of AUGUST 30th, , 1995, before me, the subscriber, personally appeared JOHN R. VOSE, to me known to be the same person described in and who executed the foregoing instrument, and he acknowledged that he executed the same.

Le soussigné, Notaire associé à LYON (Rhône)
3, Rue Président Carnot, certifie véritable les signatures et paraphes
apposés sur le présent document par : M. John VOSE.

Notary Public

Me Roland TOURNIER Notaire associé à LYON (Rhône) - COUR D'APPEL -

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

FEBRUARY 10, 1996

PTAS

100074997A

SIM & MCBURNEY
MICHAEL I. STEWART
SUITE 701, 330 UNIVERSITY AVENUE
TORONTO, ONTARIO
CANADA, M5G 1R7

UNITED STATES PATENT AND TRADEMARK OFFICE
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RECORDATION DATE: 10/03/1995

REEL/FRAME: 7645/0001
NUMBER OF PAGES: 5

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
FAHIM, RAAFAT E.F. DOC DATE: 07/05/1995

ASSIGNOR:
THIPPHAWONG, JOHN DOC DATE: 07/05/1995

ASSIGNOR:
BARRETO, LUIS DOC DATE: 07/05/1995

ASSIGNOR:
JACKSON, GAIL E.D. DOC DATE: 07/05/1995

ASSIGNOR:
TAN, LARRY U.L. DOC DATE: 07/05/1995

ASSIGNOR:
HERBERT, ANDREW DOC DATE: 07/05/1995

ASSIGNOR:
KLEIN, MICHEL H. DOC DATE: 07/05/1995

ASSIGNEE:
CONNAUGHT LABORATORIES LIMITED
1755 STEELES AVENUE WEST
WILLOWDALE, ONTARIO, CANADA M2R 3T4

SERIAL NUMBER: 08433646 FILING DATE: 05/04/1995
PATENT NUMBER: ISSUE DATE:

ANNIE HARRELL, PARALEGAL
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

10-06-1995 581 D

100074995

PTO-1595 (Rev. 6-93)

RECORDATION FORM COVER SHEET
PATENTS ONLY

MRD 10-3-95

U.S. Dept. of Commerce
Patent and Trademark Office

Honorable Commissioner
of Patents and Trademarks
Washington, D.C. 20231.

Sir:

Please record the original documents or copy thereof.

1. Name of conveying partyies: RAAFAT E.F. FAHIM, JOHN THIPPHAWONG, LUIS BARRETO, GAIL E.D. JACKSON, LARRY U.L. TAN, ANDREW HERBERT and MICHEL H. KLEIN

   Additional Names Attached? __ Yes _X_ No

2. Name and address of receiving party(ies)

   Name: CONNAUGHT LABORATORIES LIMITED

   Internal Address: _____

   Street Address: 1755 Steeles Avenue West

   City: Willowdale State: Ontario Zip: M2R 3T4

   Additional Names Attached? __ Yes _X_ No

3. Nature of Conveyance:

   _X_ Assignment
   ___ Security Agreement
   ___ Merger
   ___ Change of Name
   ___ Other _____

   Execution Date: July 5, 1995

4. Application number(s) or patent number(s):

   If this document is being filed together with a new application, the execution date of the application is: _____.

   A. Patent Application No.(s) 08/433,646

   B. Patent No.(s) _____

   Additional numbers attached? __ Yes _X_ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Michael I. Stewart
Sim & McBurney
Suite 701, 330 University Avenue
Toronto, Ontario
Canada, M5G 1R7
(416) 595-1155

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41) $40.00

X Enclosed

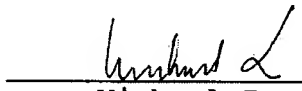
___ Authorized to be charged to Deposit Account (if necessary)

8. Deposit Account No.: ___
(Attach duplicate copy of this page if paying by deposit account)

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

October 2, 1995
Date



Michael I. Stewart
Reg. No. 24,973

Total number of pages including cover sheet, attachments, and document: 11

and set over and do hereby sell, assign, transfer and set over unto the said assignee, its successors and assigns, the entire and exclusive right, title and interest in and to said invention, patent applications and Letters Patent which may be granted or issued for said invention in the United States and throughout the world, including all divisions, continuations, reissues and extensions thereof and all international priority rights associated therewith all to be held by us had this assignment not been made, and we hereby authorize and request the Commissioner of Patents and Trademarks of the United States and the duly constituted authorities of all foreign countries of the world to issue all Letters Patent relating to the foregoing rights to said assignee, its successors and assigns;

AND we further agree that we will promptly, upon request and without further compensation, do all lawful acts including the execution of all necessary documents, and the giving of testimony that in the opinion of the said assignee, its successors and assigns may be necessary or desirable for obtaining, sustaining, or reissuing United States and foreign Letters Patent relating to the foregoing assigned rights, and for perfecting, affirming, recording and maintaining the title of said assignee, its successors and assigns thereto, and that we will generally cooperate to the fullest extent in all matters pertaining to said invention and patents, and said assignee's title thereto.

IN WITNESS WHEREOF, we have hereunto set our hands and seal this 5 day of July, 1995.

RAAFAT E. F. FAHIM

JOHN R. VOSE

JOHN THIPPHAWONG

LUIS BARRETO

GAIL E. D. JACKSON

LARRY U.L. TAN

ANDREW HERBERT

MICHEL H. KLEIN

On this 5 day of July, 1995, before me, the subscriber, personally appeared RAAFAT E. F. FAHIM, ~~JOHN R. VOSE~~, JOHN THIPPHAWONG, LUIS BARRETO, GAIL E. D. JACKSON, LARRY U.L. TAN, ANDREW HERBERT and MICHEL H. KLEIN, to me known to be the same persons described in and who executed the foregoing instrument, and they acknowledged that they executed the same.

Notary Public

GAVIN ROSS ZEALEY, Notary Public, Municipality of Metropolitan Toronto, Entitled to the attestation of instruments and the taking of affidavits, for Connaught Laboratories Limited. Expires March 15, 1998.

EXHIBIT D

AHFS Category: 80:12

DTaP-IPV/Hib

℞ only

Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine

Pentacel®

DESCRIPTION

Pentacel, [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine] (DTaP-IPV/Hib) is a vaccine for intramuscular injection. It consists of a Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus (DTaP-IPV) component and an ActHIB® vaccine component. ActHIB vaccine (Haemophilus b Conjugate Vaccine [Tetanus Toxoid Conjugate]), consists of *Haemophilus influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate [PRP]) covalently bound to tetanus toxoid (PRP-T). The DTaP-IPV component is supplied as a sterile liquid used to reconstitute the lyophilized ActHIB vaccine component to form Pentacel vaccine. Pentacel vaccine is a uniform, cloudy, white to off-white (yellow tinge) suspension.

Each 0.5 mL dose of Pentacel vaccine contains the following active ingredients:

diphtheria toxoid	15 Lf
tetanus toxoid	5 Lf
acellular pertussis antigens:	
pertussis toxin (PT) detoxified	20 µg
filamentous hemagglutinin (FHA)	20 µg
pertactin (PRN)	3 µg
fimbriae types 2 and 3 (FIM)	5 µg
inactivated polioviruses:	
Type 1 (Mahoney)	40 D-antigen units
Type 2 (MEF-1)	8 D-antigen units
Type 3 (Saukett)	32 D-antigen units
PRP of *Haemophilus influenzae* type b covalently bound to	
24 µg of tetanus toxoid (PRP-T)	10 µg

Other ingredients per 0.5 mL dose include 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, polysorbate 80 (approximately 10 ppm by calculation), ≤5 µg residual formaldehyde, <50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative) and <4 pg of neomycin and <4 pg polymyxin B sulfate.

Corynebacterium diphtheriae is grown in modified Mueller's growth medium. (1) After purification by ammonium sulfate fractionation, the diphtheria toxin is detoxified with formaldehyde and diafiltered.

Clostridium tetani is grown in modified Mueller-Miller casamino acid medium. (2) Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

The acellular pertussis vaccine antigens are produced from *Bordetella pertussis* cultures grown in Stainer-Scholte medium (3) modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. FIM are extracted and copurified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT is detoxified with glutaraldehyde. FHA is treated with formaldehyde and the residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate.

Poliovirus Type 1, Type 2 and Type 3 are each grown in separate cultures of MRC-5 cells, a line of normal human diploid cells, by the microcarrier method. (4) (5) The cells are grown in CMRL (Connaught Medical Research Laboratories) 1969 medium, supplemented with calf serum. For viral growth, the culture medium is replaced by Medium 199, without calf serum. After clarification and filtration, the viral suspensions are concentrated by ultrafiltration, and purified by liquid chromatography steps. The monovalent viral suspensions are inactivated with formaldehyde. Monovalent concentrates of each inactivated poliovirus are combined to produce a trivalent poliovirus concentrate.

The adsorbed diphtheria, tetanus and acellular pertussis antigens are combined into an intermediate concentrate. The trivalent poliovirus concentrate is added and the DTaP-IPV component is diluted to its final concentration. The DTaP-IPV component does not contain a preservative.

Both diphtheria and tetanus toxoids induce at least 2 neutralizing units per mL in the guinea pig potency test. The potency of the acellular pertussis antigens is evaluated by the antibody response of immunized mice to PT, FHA, PRN and FIM as measured by enzyme-linked immunosorbent assay (ELISA). The immunogenicity of the inactivated polioviruses is evaluated by the antibody response in monkeys measured by virus neutralization.

PRP, a high molecular weight polymer, is prepared from the *Haemophilus influenzae* type b strain 1482 grown in a semi-synthetic medium. (6) The tetanus toxoid for conjugation to PRP is prepared by ammonium sulfate purification, and formalin inactivation of the toxin from cultures of *Clostridium tetani* (Harvard strain) grown in a modified Mueller and Miller medium. (7) The toxoid is filter sterilized prior to the conjugation process. The ActHIB vaccine component does not contain a preservative. Potency of the ActHIB vaccine component is specified on each lot by limits on the content of PRP polysaccharide and protein per dose and the proportion of polysaccharide and protein that is characterized as high molecular weight conjugate.

CLINICAL PHARMACOLOGY

The efficacy of Pentacel vaccine is based on the immunogenicity of the individual antigens compared to separately administered vaccines. Serological correlates of protection exist for diphtheria, tetanus, poliomyelitis, and invasive disease due to *H influenzae* type b. The efficacy against pertussis, for which there is no well established serological correlate of protection, was based, in part, on a comparison of pertussis immune responses following Pentacel vaccine in US children to responses following DAPTACEL vaccine (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) manufactured by Sanofi Pasteur Limited) in an efficacy study conducted in Sweden (Sweden I Efficacy Trial). While Pentacel and DAPTACEL vaccines contain the same pertussis antigens, manufactured by the same process, Pentacel vaccine contains twice as much detoxified PT and four times as much FHA as DAPTACEL vaccine.

The vaccination schedules of Pentacel vaccine, Control vaccines, and concomitantly administered vaccines used in clinical studies of Pentacel vaccine that are referred to in this package insert are provided in Table 1. With the exception of Study 5A9908, which was conducted in Canada, all other studies listed in Table 1 were conducted in the US.

Table 1: Clinical Studies of Pentacel Vaccine: Vaccination Schedules

Study	Pentacel	Control Vaccines	Concomitantly Administered Vaccines
494-01	2, 4, 6 and 15 months	HCPDT + POLIOVAX + ActHIB at 2, 4, 6, and 15 months	7-valent pneumococcal conjugate vaccine* (PCV7) at 2, 4, and 6 months in a subset of participants† Hepatitis B vaccine at 2 and 6 months‡
P3T06	2, 4, 6, and 15-16 months	DAPTACEL + IPOL + ActHIB at 2, 4, and 6 months; and DAPTACEL + ActHIB at 15-16 months	PCV7* at 2, 4, and 6 months Hepatitis B vaccine at 2 and 6 months.
M5A10	2, 4, and 6 months	DAPTACEL + IPOL + ActHIB at 2, 4, and 6 months	PCV7* at 2, 4, and 6 months Hepatitis B vaccine at 2 and 6 months.
494-03	2, 4, 6, and 15-16 months	None	PCV7* at 2, 4, and 6 months in all participants; and at 15 months in a random subset of participants Hepatitis B vaccine at 2 and 6 months (if a dose was previously administered) or at 2, 4, and 6 months (if no previous dose). Measles, mumps, rubella vaccine§ (MMR) and varicella vaccine§ at 12 or 15 months in random subsets of participants
5A9908	15-18 months**	None	None

HCPDT: non-US licensed DTaP vaccine that is identical to the DTaP component of Pentacel vaccine.
POLIOVAX: US licensed Poliovirus Vaccine Inactivated, Sanofi Pasteur Limited.
IPOL: US licensed Poliovirus Vaccine Inactivated, Sanofi Pasteur SA.

* PCV7 manufactured by Wyeth Laboratories.

† PCV7 was introduced after the study was initiated, and thus, administered concomitantly with Pentacel vaccine in a subset of participants.

‡ The first dose of hepatitis B vaccine (manufacturer not specified) was administered prior to study initiation, from birth to 21 days of age [Studies 494-01, 494-03 (subset of participants), and P3T06] or from birth to 30 days prior to Dose 1 of study vaccines (Study M5A10). Subsequent doses were with hepatitis B vaccine manufactured by Merck and Co. (Studies 494-01, 494-03, and P3T06) or manufactured either by Merck and Co. or GlaxoSmithKline Biologicals (Study M5A10).

§ MMR and varicella vaccines were both manufactured by Merck and Co.

** Study participants previously had received three doses of Pentacel vaccine by 8 months of age.

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection. Antitoxin levels of at least 0.1 IU/mL are generally regarded as protective. (8) Levels of 1.0 IU/mL have been associated with long term protection. (9)

The proportions of participants achieving diphtheria antitoxin seroprotective levels one month following three and four doses of Pentacel vaccine or DAPTACEL vaccine in Study P3T06 are provided in Table 2.

Tetanus

Tetanus is an acute disease caused by an extremely potent neurotoxin produced by *C tetani*. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assay is considered the minimum protective level. (8) (10) A tetanus antitoxoid level of ≥0.1 IU/mL as measured by the ELISA used in clinical studies of Pentacel vaccine is considered protective.

The proportions of participants achieving tetanus antitoxoid seroprotective levels one month following three and four doses of Pentacel vaccine or DAPTACEL vaccine in Study P3T06 are provided in Table 2.

Table 2: Study P3T06 Diphtheria Antitoxin and Tetanus Antitoxoid Responses One Month Following Dose 3 and Dose 4 of Pentacel Vaccine or DAPTACEL + IPOL + ActHIB Vaccines in US Children Vaccinated at 2, 4, 6, and 15-16 Months of Age

	Pentacel Vaccine	DAPTACEL + IPOL + ActHIB Vaccines
Post-Dose 3	**N = 331-345**	**N = 1,037-1,099**
Diphtheria Antitoxin		
% ≥0.01 IU/mL*	100.0%	100.0%
% ≥0.10 IU/mL†	98.8%	98.5%
Tetanus Antitoxoid		
% ≥0.10 IU/mL	99.7%	100.0%
Post-Dose 4	**N = 341-352**	**N = 328-334**
Diphtheria Antitoxin		
% ≥0.10 IU/mL*	100.0%	100.0%
% ≥1.0 IU/mL	96.5%	95.7%
Tetanus Antitoxoid		
% ≥0.10 IU/mL*	100.0%	100.0%
% ≥1.0 IU/mL†‡	92.9%	99.4%

Per Protocol Immunogenicity population.

* Seroprotection rate following Pentacel vaccine is not inferior to DAPTACEL vaccine (upper limit of 90% CI of the difference DAPTACEL – Pentacel is <10%).

† Non-inferiority criteria were not pre-specified.

‡ With the ELISA used in this study, a tetanus antitoxoid level of 1.0 IU/mL is 10 times the protective level.

Pertussis

Pertussis (whooping cough) is a respiratory disease caused by *B pertussis*. This Gram-negative coccobacillus produces a variety of biologically active components, though their role in either the pathogenesis of, or immunity to, pertussis has not been clearly defined.

In a clinical pertussis vaccine efficacy study conducted in Sweden during 1992-1995 (Sweden I Efficacy Trial), 2,587 infants received DAPTACEL vaccine and 2,574 infants received a non-US licensed DT vaccine as placebo at 2, 4, and 6 months of age. (11) The mean length of follow-up was 2 years after the third dose of vaccine. The protective efficacy of DAPTACEL vaccine against pertussis after 3 doses of vaccine using the World Health Organization (WHO) case definition (≥21 consecutive days of paroxysmal cough with culture or serologic confirmation or epidemiologic link to a confirmed case) was 84.9% (95% confidence interval [CI] 80.1%, 88.6%). (12) (13) The protective efficacy of DAPTACEL vaccine against mild pertussis (≥1 day of cough with laboratory confirmation) was 77.9% (95% CI 72.6%, 82.2%). Protection against pertussis by DAPTACEL vaccine was sustained for the 2-year follow-up period.

Based on comparisons of the immune responses to DAPTACEL vaccine in US infants (Post-Dose 3) and Canadian children (Post-Dose 4) relative to infants who participated in the Sweden I Efficacy Trial, it was concluded that 4 doses of DAPTACEL vaccine were needed for primary immunization against pertussis in US children. (11)

In a serology bridging analysis, immune responses to FHA, PRN and FIM in a subset of infants who received three doses of DAPTACEL vaccine in the Sweden I Efficacy Trial were compared to the Post-Dose 3 and Post-Dose 4 responses in a subset of US children from Study 494-01 who received Pentacel vaccine (Table 3). Available stored sera from infants who received DAPTACEL vaccine in the Sweden I Efficacy Trial and sera from children who received PCV7 concomitantly with the first three doses of Pentacel vaccine in Study 494-01 (Table 1) were assayed in parallel. Data on levels of antibody to PT using an adequately specific assay were not available for this serology bridging analysis.

Geometric mean antibody concentrations (GMCs) and seroconversion rates for antibodies to FHA, PRN and FIM one month following Dose 3 of DAPTACEL vaccine in the subset of infants from the Sweden I Efficacy Trial and one month following Dose 3 and Dose 4 of Pentacel vaccine in a subset of infants from US Study 494-01 are presented in Table 3. Seroconversion was defined as 4-fold rise in antibody level (Post-Dose 3/Pre-Dose 1 or Post-Dose 4/Pre-Dose 1). For anti-FHA and anti-FIM, the non-inferiority criteria were met for seroconversion rates, and for anti-FHA, anti-PRN, and anti-FIM, the non-inferiority criteria were met for GMCs, following Dose 4 of Pentacel vaccine relative to Dose 3 of DAPTACEL vaccine. The non-inferiority criterion for anti-PRN seroconversion following Dose 4 of Pentacel vaccine relative to Dose 3 of DAPTACEL vaccine was not met [upper limit of 95% CI for difference in rate (DAPTACEL minus Pentacel) = 13.24%]. Whether the lower anti-PRN seroconversion rate following Dose 4 of Pentacel vaccine in US children relative to Dose 3 of DAPTACEL vaccine in Swedish infants correlates with diminished efficacy of Pentacel vaccine against pertussis is unknown.

Table 3: FHA, PRN and FIM Antibody Responses One Month Following Dose 3 of DAPTACEL Vaccine in a Subset of Infants Vaccinated at 2, 4, and 6 Months of Age in the Sweden I Efficacy Trial and One Month Following Dose 3 and Dose 4 of Pentacel Vaccine in a Subset of Infants Vaccinated at 2, 4, 6, and 15-16 Months of Age in US Study 494-01

	Post-Dose 3 DAPTACEL Vaccine Sweden I Efficacy Trial N = 80	Post-Dose 3 Pentacel Vaccine* US Study 494-01 N = 730-995	Post-Dose 4 Pentacel Vaccine† US Study 494-01 N = 507-554
Anti-FHA			
% achieving 4-fold rise‡	68.8	79.8	91.7§
GMC (EU/mL)	40.70	71.46	129.85§
Anti-PRN			
% achieving 4-fold rise‡	98.8	74.4	89.2**
GMC (EU/mL)	111.26	38.11	90.82§
Anti-FIM			
% achieving 4-fold rise‡	86.3	86.5	91.5§
GMC (EU/mL)-	339.31	265.02	506.57§

Analyzed sera were from subsets of the Per Protocol Immunogenicity populations in each study.
Data on anti-PT levels using an adequately specific assay were not available.

* Non-inferiority criteria were not pre-specified for the comparisons of immune responses to Pentacel vaccine Post-Dose 3 vs. DAPTACEL vaccine Post-Dose 3.

† Pre-specified non-inferiority analyses compared immune responses to Pentacel vaccine Post-Dose 4 vs. DAPTACEL vaccine Post-Dose 3.

‡ Fold rise was calculated as Post-Dose 3/Pre-Dose 1 antibody level or Post-Dose 4/Pre-Dose 1 antibody level.

§ Percent achieving 4-fold rise or GMC Post-Dose 4 Pentacel vaccine is not inferior to Post-Dose 3 DAPTACEL vaccine [upper limit of 95% CI for difference in rates (DAPTACEL minus Pentacel) <10% and upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) <1.5].

** Non-inferiority criterion is not met for percent achieving 4-fold rise in anti-PRN Post-Dose 4 Pentacel vaccine relative to Post-Dose 3 DAPTACEL vaccine [upper limit of 95% CI for difference in rates (DAPTACEL minus Pentacel) = 13.24%, exceeds the non-inferiority criterion of <10%].

In a separate study, Study P3T06, US infants were randomized to receive either Pentacel vaccine or DAPTACEL + IPOL + ActHIB vaccines at 2, 4, 6, and 15-16 months of age (Table 1). The pertussis immune responses (GMCs and seroconversion rates) one month following the third and fourth doses were compared between the two vaccine groups (Table 4). Seroconversion was defined as a 4-fold rise in antibody level (Post-Dose 3/Pre-Dose 1 or Post-Dose 4/Pre-Dose 1). Data on anti-PT responses obtained from an adequately specific assay were available on only a non-random subset of study participants. The subset of study participants was representative of all study participants with regard to Pre-Dose 1, Post-Dose 3 and Post-Dose 4 GMCs of antibodies to FHA, PRN and FIM. For each of the pertussis antigens, non-inferiority criteria were met for seroconversion rates and GMCs following Dose 3 of Pentacel vaccine relative to Dose 3 of DAPTACEL vaccine. Following Dose 4 of Pentacel vaccine relative to Dose 4 of DAPTACEL vaccine, non-inferiority criteria were met for all comparisons except for anti-PRN GMCs [upper limit of 90% CI for ratio of GMCs (DAPTACEL/Pentacel) = 2.25]. Whether the lower anti-PRN GMC following Dose 4 of Pentacel vaccine relative to Dose 4 of DAPTACEL vaccine in US children correlates with diminished efficacy of Pentacel vaccine against pertussis is unknown.

Table 4: Pertussis Antibody Responses One Month Following Doses 3 and 4 of Pentacel Vaccine or DAPTACEL + IPOL + ActHIB Vaccines in US Infants Vaccinated at 2, 4, 6, and 15-16 Months of Age in Study P3T06

	Post-Dose 3 Pentacel Vaccine	Post-Dose 3 DAPTACEL + IPOL + ActHIB Vaccines	Post-Dose 4 Pentacel Vaccine	Post-Dose 4 DAPTACEL + ActHIB Vaccines
	N = 143	N = 481-485	N = 113	N = 127-128
Anti-PT				
% achieving 4-fold rise*	95.8†	87.3	93.8‡	91.3
GMC (EU/mL)	102.62	61.88	107.89	100.29
	N = 218-318	**N = 714-1,016**	**N = 230-367**	**N = 237-347**
Anti-FHA				
% achieving 4-fold rise	81.9§	60.9	88.4**	79.3
GMC (EU/mL)	73.68	29.22	107.94	64.02
Anti-PRN				
% achieving 4-fold rise	74.2	75.4	92.7	98.3
GMC (EU/mL)	36.05	43.25	93.59††	186.07
Anti-FIM				
% achieving 4-fold rise	91.7	86.3	93.5	91.6
GMC (EU/mL)	268.15	267.18	553.39	513.54

Per Protocol Immunogenicity population for anti-FHA, anti-PRN, and anti-FIM.
Non-random subset of per Protocol Immunogenicity population for anti-PT. See text for further information on the subset evaluated.

* Fold rise was calculated as Post-Dose 3/Pre-Dose 1 antibody level or Post-Dose 4/Pre-Dose 1 antibody level.

† Percent achieving 4-fold rise or GMC Post-Dose 3 Pentacel vaccine not inferior to Post-Dose 3 DAPTACEL vaccine [upper limit of 95% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 95% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

‡ Percent achieving 4-fold rise or GMC Post-Dose 4 Pentacel vaccine not inferior to Post-Dose 4 DAPTACEL vaccine [upper limit of 95% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 95% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

§ Percent achieving 4-fold rise or GMC Post-Dose 3 Pentacel vaccine not inferior to Post-Dose 3 DAPTACEL vaccine [upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 90% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

** Percent achieving 4-fold rise or GMC Post-Dose 4 Pentacel vaccine not inferior to Post-Dose 4 DAPTACEL vaccine [upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 90% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

†† Non-inferiority criterion is not met for GMC Post-Dose 4 Pentacel vaccine relative to Post-Dose 4 DAPTACEL vaccine [upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) = 2.25, which exceeds the non-inferiority criterion of <1.5].

Poliomyelitis

Polioviruses, of which there are three serotypes (Types 1, 2, and 3) are enteroviruses. The presence of poliovirus type-specific neutralizing antibodies has been correlated with protection against poliomyelitis. (14)

In Study P3T06 (Table 1), in which infants were randomized to receive the first three doses of Pentacel vaccine or DAPTACEL + IPOL + ActHIB vaccines at 2, 4, and 6 months of age, one month following the third dose of study vaccines, ≥99.4% of participants in both groups (Pentacel: N = 338-350), (DAPTACEL + IPOL + ActHIB: N = 1,050-1,097) achieved neutralizing antibody levels of ≥1:8 for Poliovirus types 1, 2, and 3.

In Study 494-01 (Table 1), in which infants were randomized to receive Pentacel vaccine or HCPDT + POLIOVAX + ActHIB vaccines, GMTs (1/dil) of antibodies to Poliovirus types 1, 2, and 3 one month following Dose 4 of Pentacel vaccine (N = 851-857) were 2,304, 4,178, and 4,415, respectively, and one month following Dose 4 of POLIOVAX vaccine (N = 284-287) were 2,330, 2,840, and 3,300, respectively.

Invasive Disease Due to *H influenzae* Type b

H influenzae type b can cause invasive disease such as meningitis and sepsis. Anti-PRP antibody has been shown to correlate with protection against invasive disease due to *H influenzae* type b. Based on data from passive antibody studies (15) and an efficacy study with *H influenzae* type b polysaccharide vaccine in Finland, (16) a post-vaccination anti-PRP level of 0.15 µg/mL has been accepted as a minimal protective level. Data from an efficacy study with *H influenzae* type b polysaccharide vaccine in Finland indicate that a level >1.0 µg/mL 3 weeks after vaccination predicts protection through a subsequent one-year period. (17) (18) These levels have been used to evaluate the effectiveness of Haemophilus b Conjugate Vaccines, including the ActHIB vaccine component of Pentacel vaccine.

Anti-PRP seroprotection rates and GMCs one month following Dose 3 of Pentacel vaccine or separately administered ActHIB vaccine in studies P3T06 and M5A10 are presented in Table 5. In Study 494-01, non-inferiority criteria were not met for the proportion of participants who achieved an anti-PRP level ≥1.0 µg/mL and for anti-PRP GMCs following Pentacel vaccine compared with separately administered ActHIB vaccine. In each of Studies P3T06 and M5A10, the non-inferiority criterion was met for the proportion of participants who achieved an anti-PRP level ≥1.0 µg/mL following Pentacel vaccine compared with separately administered ActHIB vaccine. In Study M5A10, the non-inferiority criterion was met for anti-PRP GMCs following Pentacel vaccine compared with separately administered ActHIB vaccine.

Table 5: Anti-PRP Seroprotection Rates and GMCs One Month Following Three Doses of Pentacel Vaccine or Separate DTaP + IPV + ActHIB Vaccines Administered at 2, 4, and 6 Months of Age in Studies 494-01, P3T06, and M5A10

	Study 494-01	
	Pentacel Vaccine N = 1,127	**HCPDT + POLIOVAX + ActHIB Vaccines N = 401**
% achieving anti-PRP ≥0.15 µg/mL	95.4*	98.3
% achieving anti-PRP ≥1.0 µg/mL	79.1†	88.8
Anti-PRP GMC (µg/mL)	3.19‡	6.23
	Study P3T06	
	Pentacel Vaccine N = 365	**DAPTACEL + IPOL + ActHIB Vaccines N = 1,128**
% achieving anti-PRP ≥0.15 µg/mL	92.3*	93.3
% achieving anti-PRP ≥1.0 µg/mL	72.1*	70.8
Anti-PRP GMC (µg/mL)	2.31§	2.29
	Study M5A10	
	Pentacel Vaccine N = 826	**DAPTACEL + IPOL + ActHIB Vaccines N = 421**
% achieving anti-PRP ≥0.15 µg/mL	93.8**	90.3
% achieving anti-PRP ≥1.0 µg/mL	75.1**	74.8
Anti-PRP GMC (µg/mL)	2.52††	2.38

Per Protocol Immunogenicity population for all studies.

IPV indicates Poliovirus Vaccine Inactivated.

* Percent achieving specified level following Pentacel vaccine not inferior to ActHIB vaccine [upper limit of 90% CI for difference in rates (ActHIB minus Pentacel) <10%].

† Non-inferiority criterion not met for percent achieving anti-PRP ≥1.0 µg/mL following Pentacel vaccine relative to ActHIB vaccine [upper limit of 90% CI for difference in rates (ActHIB minus Pentacel), 12.9%, exceeds the non-inferiority criterion <10%].

‡ Non-inferiority criterion not met for GMC following Pentacel vaccine relative to ActHIB vaccine [upper limit of 90% CI of GMC ratio (ActHIB/Pentacel), 2.26, exceeds the non-inferiority criterion <1.5].

§ Non-inferiority criterion not pre-specified.

** Percent achieving specified level following Pentacel vaccine not inferior to ActHIB vaccine [upper limit of 95% CI for difference in rates (ActHIB minus Pentacel) <10%].

†† GMC following Pentacel vaccine not inferior to ActHIB vaccine [upper limit of 90% CI of GMC ratio (ActHIB/Pentacel) <1.5].

In Study 494-01, at 15 months of age prior to receipt of Dose 4 of study vaccines, 68.6% of Pentacel vaccine recipients (N = 829) and 80.8% of separately administered ActHIB vaccine recipients (N = 276) had an anti-PRP level ≥0.15 µg/mL. Following Dose 4 of study vaccines, 98.2% of Pentacel vaccine recipients (N = 874) and 99.0% of separately administered ActHIB vaccine recipients (N = 291) had an anti-PRP level ≥1.0 µg/mL.

In Study P3T06, at 15 months of age prior to receipt of Dose 4 of study vaccines, 65.4% of Pentacel vaccine recipients (N = 335) and 60.7% of separately administered ActHIB vaccine recipients (N = 323) had an anti-PRP level ≥0.15 µg/mL. Following Dose 4 of study vaccines, 97.8% of Pentacel vaccine recipients (N = 361) and 95.9% of separately administered ActHIB vaccine recipients (N = 340) had an anti-PRP level ≥1.0 µg/mL.

Concomitantly Administered Vaccines

Vaccines administered concomitantly with Pentacel vaccine in clinical trials are listed in Table 1.

In Study P3T06, there was no evidence for reduced antibody responses to hepatitis B vaccine (percent of participants with anti-HBsAg ≥10 mIU/mL and GMCs) or PCV7 (percent of participants with antibody levels ≥0.15 µg/mL and ≥0.5 µg/mL and GMCs to each serotype) administered concomitantly with Pentacel vaccine (N = 321-325) relative to these vaccines administered concomitantly with DAPTACEL + IPOL + ActHIB vaccines (N = 998-1,029). The immune responses to hepatitis B vaccine and PCV7 were evaluated one month following the third dose.

In Study 494-03, there was no evidence for interference in the immune response to the fourth dose of PCV7 (percent of participants with antibody levels ≥0.15 µg/mL and ≥0.5 µg/mL and GMCs to each serotype) administered at 15 months of age concomitantly with Pentacel vaccine (N = 155) relative to this vaccine administered concomitantly with MMR and varicella vaccines (N = 158). There was no evidence for interference in the immune response to MMR and varicella vaccines (percent of participants with pre-specified seroresponse level) administered at 15 months of age concomitantly with Pentacel vaccine (N = 154) relative to these vaccines administered concomitantly with PCV7 (N = 144). The immune responses to MMR, varicella vaccine and the fourth dose of PCV7 were evaluated one month post-vaccination.

INDICATIONS AND USAGE

Pentacel vaccine is indicated for active immunization against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease due to *Haemophilus influenzae* type b. Pentacel vaccine is approved for use in children 6 weeks through 4 years of age (prior to fifth birthday).

CONTRAINDICATIONS

A severe allergic reaction (e.g., anaphylaxis) after a previous dose of Pentacel vaccine, any ingredient of this vaccine, or any other tetanus toxoid, diphtheria toxoid, pertussis-containing vaccine, inactivated poliovirus vaccine or *H influenzae* type b vaccine is a contraindication to administration of Pentacel vaccine. (See DESCRIPTION). Because of uncertainty as to which ingredient of the vaccine may be responsible, none of the ingredients should be administered. Alternatively, such individuals may be referred to an allergist for evaluation if further immunizations are considered.

The following events are contraindications to administration of any pertussis-containing vaccine, (19) including Pentacel vaccine.

- Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of a previous dose of a pertussis containing vaccine that is not attributable to another identifiable cause.

- Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, progressive encephalopathy. Pertussis vaccine should not be administered to individuals with such conditions until the neurologic status is clarified and stabilized.

WARNINGS

If any of the following events occur within the specified period after administration of a whole-cell pertussis or acellular pertussis-containing vaccine, the decision to administer Pentacel vaccine or any pertussis-containing vaccine should be based on careful consideration of potential benefits and possible risks. (19) (See DOSAGE AND ADMINISTRATION.)

- Temperature of ≥40.5°C (≥105°F) within 48 hours, not attributable to another identifiable cause.
- Collapse or shock-like state (hypotonic-hyporesponsive episode [HHE]) within 48 hours.
- Persistent, inconsolable crying lasting ≥3 hours within 48 hours.
- Seizure with or without fever within 3 days.

A review by the Institute of Medicine (IOM) found evidence for a causal relation between tetanus toxoid and brachial neuritis, Guillain-Barré syndrome and anaphylaxis. (20) If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give Pentacel vaccine or any vaccine containing tetanus toxoid should be based on careful consideration of the potential benefits and possible risks. (19)

Vaccination with Pentacel vaccine may not protect all individuals.

PRECAUTIONS

General

Before administration of Pentacel vaccine, the patient's current health status and medical history should be reviewed in order to determine whether any contraindications exist and to assess the benefits and risks of vaccination. (See CONTRAINDICATIONS and WARNINGS).

Epinephrine Hydrochloride Solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

For infants or children at higher risk for seizures than the general population, an appropriate antipyretic may be administered (in the dosage recommended in its prescribing information) at the time of vaccination with an acellular pertussis-containing vaccine (including Pentacel vaccine) and for the following 24 hours, to reduce the possibility of post-vaccination fever. (19)

If Pentacel vaccine is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained.

Information for Vaccine Recipients and Parents/Guardians

Before administration of Pentacel vaccine, health-care personnel should inform the parent or guardian of the benefits and risks of the vaccine and the importance of completing the immunization series unless a contraindication to further immunization exists.

The health-care provider should inform the parent or guardian about the potential for adverse reactions that have been temporally associated with Pentacel vaccine or other vaccines containing similar ingredients. The health-care provider should provide the Vaccine Information Statements (VIS), which are required by the National Childhood Vaccine Injury Act of 1986 to be given with each immunization. The parent or guardian should be instructed to report adverse reactions to their health-care provider.

Drug Interactions

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to Pentacel vaccine.

Drug/Laboratory Test Interactions

Antigenuria has been detected in some instances following receipt of ActHIB vaccine. Urine antigen detection may not have definite diagnostic value in suspected *H influenzae* type b disease within one week following receipt of Pentacel vaccine. (21)

Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been performed with Pentacel vaccine to evaluate carcinogenicity, mutagenic potential, or impairment of fertility.

Pregnancy Category C

Animal reproduction studies have not been conducted with Pentacel vaccine. It is not known whether Pentacel vaccine can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Pentacel vaccine is not approved for use in women of childbearing age.

Pediatric Use

The safety and effectiveness of Pentacel vaccine was established in the age group 6 weeks through 18 months on the basis of clinical studies. (See ADVERSE REACTIONS and CLINICAL PHARMACOLOGY.) The safety and effectiveness of Pentacel vaccine in the age group 19 months through 4 years is supported by evidence in children 6 weeks through 18 months. The safety and effectiveness of Pentacel vaccine in infants less than 6 weeks of age and in children 5 to 16 years of age have not been established.

Pentacel vaccine is not approved for use in persons 5 years of age or older.

Geriatric Use

Pentacel vaccine is not approved for use in adult populations.

ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events.

In Studies 494-01, 494-03, 5A9908, and P3T06 (Table 1), a total of 5,980 participants received at least one dose of Pentacel vaccine, including 4,198 participants who were enrolled in one of three US studies that evaluated the safety of four consecutive doses of Pentacel vaccine administered at 2, 4, 6, and 15-16 months of age. In calculating event rates across doses and studies, one subject who received one dose of Pentacel vaccine followed by three doses of Control vaccines was included in the control group. Two of the US studies, Study 494-01 and Study P3T06, included a control group that received separately administered vaccines. In Study 5A9908 conducted in Canada, 1,782 participants previously vaccinated with three doses of Pentacel vaccine received a fourth dose at 15-18 months of age. Across the four studies, 50.8% of participants were female. Among participants in the three US studies, 64.5% were Caucasian, 9.2% were Black, 12.9% were Hispanic, 3.9% were Asian, and 9.5% were of other racial/ethnic groups. In the two controlled studies, the racial/ethnic distribution of participants who received Pentacel and Control vaccines was similar. In the Canadian fourth dose study, 86.0% of participants were Caucasian, 1.9% were Black, 0.8% were Hispanic, 4.3% were Asian, 2.0% were East Indian, 0.5% were Native Indian, and 4.5% were of other racial/ethnic groups.

Solicited Adverse Reactions

The incidence and severity of selected solicited injection site and systemic adverse reactions that occurred within 3 days following each dose of Pentacel or Control vaccines in Study P3T06 is shown in Table 6. Information on these reactions was recorded daily by parents or guardians on diary cards. In Table 6, injection site reactions are reported for the Pentacel vaccine and DAPTACEL vaccine injection sites.

Table 6: Number (Percentage) of Children with Selected Solicited Injection Site Reactions and Solicited Systemic Adverse Events by Severity Occurring within 0-3 days of Pentacel Vaccine or Control Vaccines in Study P3T06

Injection Site Reactions	Pentacel Vaccine				DAPTACEL Vaccine			
	Dose 1 N = 465-467 %	Dose 2 N = 451 %	Dose 3 N = 438-440 %	Dose 4 N = 387-396 %	Dose 1 N = 1,400-1,404	Dose 2 N = 1,358-1,359	Dose 3 N = 1,311-1,312	Dose 4 N = 376-380 %
Redness								
>5 mm	7.1	8.4	8.7	17.3	6.2	7.1	9.6	16.4
>25 mm	2.8	1.8	1.8	9.2	1.0	0.6	1.9	7.9
>50 mm	0.6	0.2	0.0	2.3	0.4	0.1	0.0	2.4
Swelling								
>5 mm	7.5	7.3	5.0	9.7	4.0	4.0	6.5	10.3
>25 mm	3.0	2.0	1.6	3.8	1.6	0.7	1.1	4.0
>50 mm	0.9	0.0	0.0	0.8	0.4	0.1	0.1	1.3
Tenderness*								
Any	47.5	39.2	42.7	56.1	48.8	38.2	40.9	51.1
Moderate or Severe	19.6	10.6	11.6	16.7	20.7	12.2	12.3	15.8
Severe	5.4	1.6	1.4	3.3	4.1	2.3	1.7	2.4
Increase in Arm Circumference	–	–	–	33.6	–	–	–	30.6
>5 mm				4.7				6.9
>20 mm				0.5				0.8
>40 mm								

Systemic Reactions	Pentacel Vaccine				DAPTACEL + IPOL + ActHIB Vaccines			DAPTACEL + ActHIB Vaccines
	Dose 1 N = 466-467 %	Dose 2 N = 451-452 %	Dose 3 N = 435-440 %	Dose 4 N = 389-398 %	Dose 1 N = 1,390-1,406 %	Dose 2 N = 1,346-1,360 %	Dose 3 N = 1,301-1,312 %	Dose 4 N = 379-381 %
Fever†‡								
≥38.0°C	5.8	10.9	16.3	13.4	9.3	16.1	15.8	8.7
>38.5°C	1.3	2.4	4.4	5.1	1.6	4.3	5.1	3.2
>39.5°C	0.4	0.0	0.7	0.3	0.1	0.4	0.3	0.8
Decreased Activity/Lethargy§	45.8	32.7	32.5	24.1	51.1	37.4	33.2	24.1
Any	22.9	12.4	12.7	9.8	24.3	15.8	12.7	9.2
Moderate or Severe	2.1	0.7	0.2	2.5	1.2	1.4	0.6	0.3
Severe								
Inconsolable Crying								
Any	59.3	49.8	47.3	35.9	58.5	51.4	47.9	36.2
≥1 hour	19.7	10.6	13.6	11.8	16.4	16.0	12.2	10.5
>3 hours	1.9	0.9	1.1	2.3	2.2	3.4	1.4	1.8
Fussiness/Irritability								
Any	76.9	71.2	68.0	53.5	75.8	70.7	67.1	53.8
≥1 hour	34.5	27.0	26.4	23.6	33.3	30.5	26.2	19.4
>3 hours	4.3	4.0	5.0	5.3	5.6	5.5	4.3	4.5

* Any: Mild, Moderate or Severe; Mild: subject whimpers when site is touched; Moderate: subject cries when site is touched; Severe: subject cries when leg or arm is moved.

† Fever is based upon actual temperatures recorded with no adjustments to the measurement route.

‡ Following Doses 1-3 combined, the proportion of temperature measurements that were taken by axillary, rectal or other routes, or not recorded were 46.0%, 53.0%, 1.0%, and 0% respectively, for Pentacel vaccine and 44.8%, 54.0%, 1.0%, and 0.1%, respectively, for DAPTACEL + IPOL + ActHIB vaccines. Following Dose 4, the proportion of temperature measurements that were taken by axillary, rectal or other routes, or not recorded were 62.7%, 34.4%, 2.4% and 0.5%, respectively, for Pentacel vaccine, and 61.1%, 36.6%, 1.7% and 0.5%, respectively, for DAPTACEL + ActHIB vaccines.

§ Moderate: interferes with or limits usual daily activity; Severe: disabling, not interested in usual daily activity.

Hypotonic Hyporesponsive Episodes

In Study P3T06, the diary cards included questions pertaining to HHEs. In Studies 494-01, 494-03, and 5A9908, a question about the occurrence of fainting or change in mental status was asked during post-vaccination phone calls. Across these 4 studies, no HHEs, as defined in a report of a US Public Health Service workshop (22) were reported among participants who received Pentacel vaccine (N = 5,979), separately administered HCPDT + POLIOVAX + ActHIB vaccines (N = 1,032) or separately administered DAPTACEL + IPOL + ActHIB vaccines (N = 1,455). Hypotonia not fulfilling HHE criteria within 7 days following vaccination was reported in 4 participants after the administration of Pentacel vaccine (1 on the same day as the 1st dose; 3 on the same day as the 3rd dose) and in 1 participant after the administration of DAPTACEL + IPOL + ActHIB vaccines (4 days following the 1st dose).

Seizures

Across Studies 494-01, 494-03, 5A9908 and P3T06, a total of 8 participants experienced a seizure within 7 days following either Pentacel vaccine (4 participants; N = 4,197 for at least one of Doses 1-3; N = 5,033 for Dose 4), separately administered HCPDT + POLIOVAX + ActHIB vaccines (3 participants; N = 1,032 for at least one of Doses 1-3, N = 739 for Dose 4), separately administered DAPTACEL + IPOL + ActHIB vaccines (1 participant; N = 1,455 for at least one of Doses 1-3), or separately administered DAPTACEL + ActHIB vaccines (0 participants; N = 418 for Dose 4). Among the four participants who experienced a seizure within 7 days following Pentacel vaccine, one participant in Study 494-01 had an afebrile seizure 6 days after the first dose, one participant in Study 494-01 had a possible seizure the same day as the third dose, and two participants in Study 5A9908 had a febrile seizure 2 and 4 days, respectively, after the fourth dose. Among the four participants who experienced a seizure within 7 days following Control vaccines, one participant had an afebrile seizure the same day as the first dose of DAPTACEL + IPOL + ActHIB vaccines, one participant had an afebrile seizure the same day as the second dose of HCPDT + POLIOVAX + ActHIB vaccines, and two participants had a febrile seizure 6 and 7 days, respectively, after the fourth dose of HCPDT + POLIOVAX + ActHIB vaccines.

Serious Adverse Events

In Study P3T06, within 30 days following any of Doses 1-3 of Pentacel or Control vaccines, 19 of 484 (3.9%) participants who received Pentacel vaccine and 50 of 1,455 (3.4%) participants who received DAPTACEL + IPOL + ActHIB vaccines experienced a serious adverse event. Within 30 days following Dose 4 of Pentacel or Control vaccines, 5 of 431 (1.2%) participants who received Pentacel vaccine and 4 of 418 (1.0%) participants who received DAPTACEL + ActHIB vaccines experienced a serious adverse event. In Study 494-01, within 30 days following any of Doses 1-3 of Pentacel or Control vaccines, 23 of 2,506 (0.9%) participants who received Pentacel vaccine and 11 of 1,032 (1.1%) participants who received HCPDT + POLIOVAX + ActHIB vaccines experienced a serious adverse event. Within 30 days following Dose 4 of Pentacel or Control vaccines, 6 of 1,862 (0.3%) participants who received Pentacel vaccine and 2 of 739 (0.3%) participants who received HCPDT + POLIOVAX + ActHIB vaccines experienced a serious adverse event.

Across Studies 494-01, 494-03 and P3T06, within 30 days following any of Doses 1-3 of Pentacel or Control vaccines, overall, the most frequently reported serious adverse events were bronchiolitis, dehydration, pneumonia and gastroenteritis. Across Studies 494-01, 494-03, 5A9908 and P3T06, within 30 days following Dose 4 of Pentacel or Control vaccines, overall, the most frequently reported serious adverse events were dehydration, gastroenteritis, asthma, and pneumonia.

Across Studies 494-01, 494-03, 5A9908 and P3T06, two cases of encephalopathy were reported, both in participants who had received Pentacel vaccine (N = 5,979). One case occurred 30 days post-vaccination and was secondary to cardiac arrest following cardiac surgery. One infant who had onset of neurologic symptoms 8 days post-vaccination was subsequently found to have structural cerebral abnormalities and was diagnosed with congenital encephalopathy.

A total of 5 deaths occurred during Studies 494-01, 494-03, 5A9908 and P3T06: 4 in children who had received Pentacel vaccine (N = 5,979) and one in a participant who had received DAPTACEL + IPOL + ActHIB vaccines (N = 1,455). There were no deaths reported in children who received HCPDT + POLIOVAX + ActHIB vaccines (N = 1,032). Causes of death among children who received Pentacel vaccine were asphyxia due to suffocation, head trauma,

Sudden Infant Death syndrome, and neuroblastoma (8, 23, 52 and 256 days post-vaccination, respectively). One participant with ependymoma died secondary to aspiration 222 days following DAPTACEL + IPOL + ActHIB vaccines.

Data From Post-Marketing Experience

The following additional adverse events have been spontaneously reported between 1997 and 2007 during the post-marketing use of Pentacel vaccine outside of the US, primarily in Canada. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure. The following adverse events were included based on severity, frequency of reporting, or the strength of causal association to Pentacel vaccine.

- ***Cardiac disorders***

  Cyanosis

- ***Gastrointestinal disorders***

  Vomiting, diarrhea

- ***General disorders and administration site conditions***

  Injection site reactions (including inflammation, mass, abscess and sterile abscess), extensive swelling of the injected limb (including swelling that involved adjacent joints), vaccination failure/therapeutic response decreased (invasive *H influenzae* type b disease)

- ***Immune system disorders***

  Hypersensitivity (such as rash and urticaria)

- ***Infections and infestations***

  Meningitis, rhinitis, viral infection

- ***Metabolism and nutrition disorders***

  Decreased appetite

- ***Nervous system disorders***

  Somnolence, HHE, depressed level of consciousness

- ***Psychiatric disorders***

  Screaming

- ***Respiratory, thoracic and mediastinal disorders***

  Apnea, cough

- ***Skin and subcutaneous tissue disorders***

  Erythema, skin discoloration

- ***Vascular disorders***

  Pallor

Reporting of Adverse Events

The National Childhood Vaccine Injury Act of 1986 requires physicians and other health-care providers who administer vaccines to maintain in the recipient's permanent medical record the manufacturer, lot number, date of administration, and the name, address and title of the person administering the vaccine. The Act further requires the health-care provider to report to the US Department of Health and Human Services the occurrence of certain adverse events following immunization. For Pentacel vaccine, events required to be reported are anaphylaxis or anaphylactic shock within 7 days, brachial neuritis within 2-28 days, encephalopathy or encephalitis within 7 days following vaccination, or any acute complication or sequela (including death) of these events, or any contraindicating event listed in this Pentacel vaccine package insert. (23) (24) These events and other suspected adverse reactions should be reported to VAERS at 1-800-822-7967 or http://www.vaers.hhs.gov and to Sanofi Pasteur Inc. at 1-800-822-2463.

DOSAGE AND ADMINISTRATION

Vaccination Schedule

Pentacel vaccine is approved for administration as a 4 dose series at 2, 4 and 6, and 15-18 months of age. The first dose may be given as early as 6 weeks of age. Four doses of Pentacel vaccine constitute a primary immunization course against pertussis. Three doses of Pentacel vaccine constitute a primary immunization course against diphtheria, tetanus, *H influenzae* type b invasive disease, and poliomyelitis; the fourth dose constitutes a booster vaccination against diphtheria, tetanus, *H influenzae* type b invasive disease, and poliomyelitis (See CLINICAL PHARMACOLOGY.)

If a decision is made to withhold pertussis vaccine, (see CONTRAINDICATIONS and WARNINGS), vaccination against diphtheria, tetanus, poliomyelitis and invasive disease due to *H influenzae* type b should be provided.

Children who have completed a four-dose series with Pentacel vaccine should receive a fifth dose of DTaP vaccine at 4-6 years of age. Because the pertussis antigens in DAPTACEL vaccine are the same as those in Pentacel vaccine (although with different amounts of detoxified PT and FHA), these children should receive DAPTACEL vaccine as their fifth dose of DTaP. However, data are not available to evaluate the safety of DAPTACEL vaccine following four previous doses of Pentacel vaccine.

Children Previously Vaccinated With One or More Doses of DAPTACEL Vaccine:
Pentacel vaccine may be used to complete the first 4 doses of the DTaP series in infants and children who have received 1 or more doses of DAPTACEL vaccine and are also scheduled to receive the other antigens of Pentacel vaccine. However, the safety and efficacy of Pentacel vaccine in such infants have not been evaluated.

Children Previously Vaccinated With One or More Doses of IPV: Pentacel vaccine may be used to complete the 4 dose IPV series in infants and children who have received 1 or more doses of another licensed IPV vaccine and are also scheduled to receive the other antigens of Pentacel vaccine. However, the safety and efficacy of Pentacel vaccine in such infants have not been evaluated.

Children Previously Vaccinated With One or More Doses of Haemophilus b Conjugate Vaccine: Pentacel vaccine may be used to complete the vaccination series in infants and children previously vaccinated with one or more doses of a Haemophilus b Conjugate Vaccine (either separately administered or as part of another combination vaccine), who are also scheduled to receive the other antigens of Pentacel vaccine. However, the safety and efficacy of Pentacel vaccine in such infants have not been evaluated. If different brands of Haemophilus b Conjugate Vaccines are administered to complete the series, three primary immunizing doses are needed, followed by a booster dose.

Administration

Pentacel vaccine should be inspected visually for extraneous particulate matter and/or discoloration before administration. (See DESCRIPTION.) If these conditions exist, Pentacel vaccine should not be administered.

Reconstitution of Freeze-Dried Product and Withdrawal from Stoppered Vial

Thoroughly but gently shake the vial of DTaP-IPV component, withdraw the entire liquid content and inject into the vial of the lyophilized ActHIB vaccine component. Shake the vial now containing Pentacel vaccine thoroughly until a cloudy, uniform suspension results. Withdraw and administer a 0.5 mL dose of Pentacel vaccine intramuscularly. Pentacel vaccine should be used immediately after reconstitution. Refer to Figures 1, 2, 3, 4 and 5.

Pentacel Vaccine: Instructions For Reconstitution of ActHIB Vaccine Component With DTaP-IPV Component

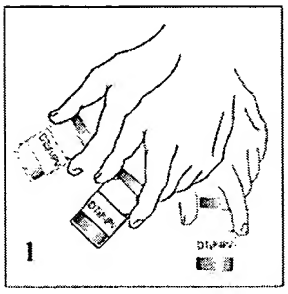


Figure 1
Gently shake the vial of DTaP-IPV component.

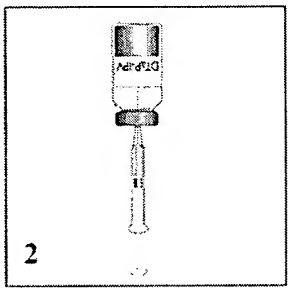


Figure 2
Withdraw the entire liquid content.

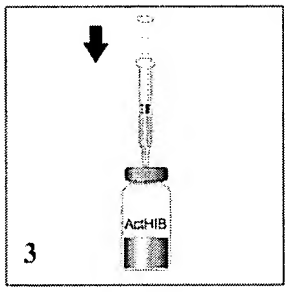


Figure 3
Insert the syringe needle through the stopper of the vial of lyophilized ActHIB vaccine component and inject the liquid into the vial.

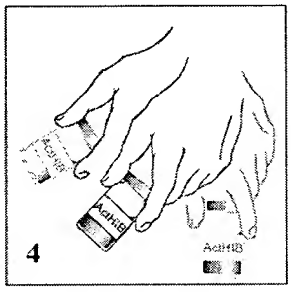


Figure 4
Shake vial thoroughly.

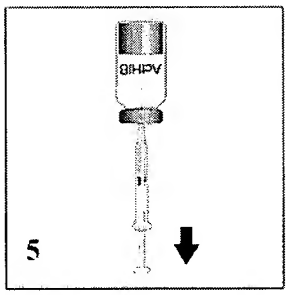


Figure 5
After reconstitution, immediately withdraw 0.5 mL of Pentacel vaccine and administer intramuscularly.
Pentacel vaccine should be used immediately after reconstitution.

In infants younger than 1 year, the anterolateral aspect of the thigh provides the largest muscle and is the preferred site of injection. In older children, the deltoid muscle is usually large enough for injection. The vaccine should not be injected into the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously or subcutaneously.

Concomitant Administration with Other Vaccines

In clinical trials, Pentacel vaccine was routinely administered, at separate sites, concomitantly with one or more of the following vaccines: hepatitis B vaccine, 7-valent pneumococcal conjugate vaccine, MMR and varicella vaccines. (See CLINICAL PHARMACOLOGY and ADVERSE REACTIONS.) When Pentacel vaccine is given at the same time as another injectable vaccine(s), the vaccines should be given with different syringes.

HOW SUPPLIED

5 Dose Package containing 5 vials of DTaP-IPV Component to be used to reconstitute five single dose vials of lyophilized ActHIB vaccine component - Product No. 49281-510-05.

STORAGE

Store at 2° to 8°C (35° to 46°F). Do not freeze. Discard product if exposed to freezing.

Do not use after expiration date.

Pentacel vaccine should be used immediately after reconstitution.

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Product information as of June 2008.

Printed in Canada.

Manufactured by:

Sanofi Pasteur Limited

Toronto Ontario Canada

and **Sanofi Pasteur SA**

Lyon France

Distributed by:

Sanofi Pasteur Inc.

Swiftwater PA 18370 USA

R0-0608 USA

sanofi pasteur

EXHIBIT E

DEPARTMENT OF HEALTH AND HUMAN SERVICES
UNITED STATES PUBLIC HEATH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVAULATION AND RESEARCH
OFFICE OF VACCINES RESEARCH AND REVIEW
DIVISION OF VACCINES AND RELATED PRODUCTS APPLICATIONS

20-06-08P03:35 RCVD

Number of Pages Faxed (including the cover sheet): 3.30

Date: June 20. 2008 Time: ~ 3:35 pm

To: Yatika Kohli

FAX Number: 416-667-2912 Phone Number:

MESSAGE: AP letter as discussed
125145/0

From: Theresa Finn.

Facsimile Numbers: 301-827-3532 (Main) or 301-827-3075 (2nd)
Telephone Number: 301-827-3070

Address: Woodmont Office Center I
1401 Rockville Pike
HFM-475, Suite 370 North
Rockville, MD 20852-1448

SBNN folder: DVRPA\WDrive

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852-1448

Our STN: BL 125145/0

JUN 20 2008

Sanofi Pasteur Limited
Attention: Gary K. Chikami, M.D.
Associate Vice President, Regulatory Affairs, North America
Sanofi Pasteur. Inc.
Discovery Drive
Swiftwater, PA 18370-0187

Dear Dr. Chikami:

We have approved your biologics license application (BLA) for Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine, effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine under your existing Department of Health and Human Services U.S. License No. 1726. Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine is indicated for active immunization against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease caused by *Haemophilus influenzae* type b when administered to infants and children 6 weeks through 4 years of age (prior to fifth birthday).

Under this license, you are approved to manufacture and fill the Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus (DTaP-IPV) component at Sanofi Pasteur, Limited, in Toronto, Canada. The Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate), ActHIB® component, is manufactured, filled and lyophilized at Sanofi Pasteur, S.A., France. Vials are labeled and packaged at Sanofi Pasteur, Limited, in Toronto, Canada. You may label your product with the proprietary name Pentacel®. The vaccine will be supplied as five dose packages containing five single dose vials of the DTaP-IPV component to be used to reconstitute five single dose vials of the lyophilized ActHIB® vaccine component.

The dating period for the DTaP-IPV component is no more than 30 months from the date of formulation of the final bulk when stored at 2 to 8 °C (35 to 46 °F). The dating period for the lyophilized ActHIB® component is 36 months when stored at 2 to 8 °C (35 to 46 °F). The dating period for the co-packaged DTaP-IPV component and lyophilized ActHIB® component shall be no more than 30 months or whichever vial has the earliest expiration date when stored at 2 to 8 °C (35 to 46 °F).

Please submit final container samples of the product in final containers together with protocols showing results of all applicable tests. You may not distribute any lots of product until you

receive a notification of release from the Director, Center for Biologics Evaluation and Research (CBER).
You must submit information to your BLA for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Pentacel®, or in the manufacturing facilities.

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Director, Office of Compliance and Biologics Quality, Center for Biologics Evaluation and Research, HFM-600, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please submit all final printed labeling at the time of use and include implementation information on FDA Form 356h and FDA Form 2567 as appropriate. Please provide a PDF-format electronic version of the label.

In addition, you may wish to submit two draft copies of the proposed introductory advertising and promotional labeling with an FDA Form 2253 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Branch, HFM-602, 1401 Rockville Pike, Rockville, MD 20852-1448. Two copies of final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by FDA Form 2253. All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have submitted data to support such claims to us and have received CBER approval for such claims.

ADVERSE EVENT REPORTING

As required under 21 CFR 600.80(c)(2) you must provide a Quarterly Periodic Adverse Experience Report to the VAERS contractor. We have granted your waiver (21 CFR 600.90) to replace the Periodic Adverse Experience Reports format with the Periodic Safety Update Report (PSUR) format as specified in the International Conference on Harmonisation (ICH) E2C guideline using the international birth date (IBD) for the product (May 12, 1997) in lieu of the date of issuance of this biologics license. As described under 21 CFR 600.80(c)(2) you must report each adverse experience not reported under paragraph (c)(1)(i) of this section in PSUR format at quarterly intervals for the first 3 years following May 12, 2008, and then at annual intervals. In addition, distribution reports are also required as described in 21 CFR 600.81. Since your product is characterized as a vaccine, submit these reports to the Vaccine Adverse Event Reporting System (VAERS) using the pre-addressed form VAERS-1.

PEDIATRIC REQUIREMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of

administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication(s) in pediatric patients unless this requirement is waived, deferred or inapplicable.

We are waiving the pediatric study requirement for this application for ages 0-5 weeks (i.e., before age 6 weeks) and 5-16 years (i.e., 5 years to prior to 17th birthday) because:

A) In the pediatric population 0-5 weeks of age, necessary studies are impossible or highly impracticable because neither diphtheria, tetanus, nor poliomyelitis occur in U.S. infants too young to be protected from vaccination beginning at 6 weeks of age.

B) In the pediatric population 0-5 weeks of age, Pentacel® does not represent a meaningful therapeutic benefit over initiating vaccination against diphtheria, tetanus, and poliomyelitis at 6 weeks of age and is not likely to be used in a substantial number of pediatric patients ages 0–5 weeks.

C) In the pediatric population 5-16 years of age, necessary studies are impossible or highly impracticable because too few children, geographically dispersed, would need vaccination with all of the antigens contained in Pentacel®.

D) In the pediatric population 10-16 years of age, Pentacel® does not represent a meaningful therapeutic benefit over vaccination with existing vaccines (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed; and Poliovirus Vaccine Inactivated) and is not likely to be used in a substantial number of patients.

We note that you have fulfilled the pediatric study requirement for infants and children 6 weeks through 4 years of age (prior to fifth birthday).

POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS OF 21 CFR 601.70

We acknowledge your written commitment to provide the following information as described in your May 27, 2008, letter as outlined below:

1. To submit clinical data to support use of DAPTACEL® to complete the DTaP series following four previous doses of Pentacel®.
   Final report submission (P3T10): July 31, 2008

We request that you submit clinical protocols and nonclinical toxicology protocols to your IND, with a cross-reference letter to this BLA, STN BL 125145/0.

Please use the following designators to label prominently all submissions, including supplements, relating to this postmarketing study commitment as appropriate:

- **Postmarketing Study Commitment Protocol**
- **Postmarketing Study Correspondence/Status Update**

- **Postmarketing Study Commitment – Final Study Report**
- **Supplement Contains Postmarketing Study Commitments – Final Study Report**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. Label your annual report "Annual Status Report of Postmarketing Study Commitments." The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e., pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e., number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding this postmarketing commitment on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cber/gdlns/post130.htm) for further information.

POSTMARKETING COMMITMENTS NOT SUBJECT TO REPORTING REQUIREMENTS OF 21 CFR 601.70

We also acknowledge your written commitments as described in your submission of May 27, 2008, and June 19, 2008, as outlined below:

2. A reanalysis of sera from Study 494-01 to support lot consistency of the inactivated pertussis toxin (PT) antigen of Pentacel®.
   Final report submission: December 31, 2008

3. A reanalysis of sera from Study 494-03 to assess the response to the inactivated PT antigen of Pentacel® when the fourth dose of Pentacel® is administered with MMR®II and VARIVAX®.
   Final report submission: December 31, 2008

4. To submit assay validation and acceptance specifications for the revised PT, FHA and FIM component pertussis ELISA Immunogenicity assay. A supplement for this change will be submitted by September 26, 2008.

We request that you submit information concerning nonclinical and chemistry, manufacturing, and control postmarketing commitments and final reports to your BLA, STN BL125145/0.

Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- Postmarketing Correspondence
- Postmarketing Commitment – Final Study Report
- Supplement Contains Postmarketing Commitment – Final Study Report

For each postmarketing commitment not subject to the reporting requirements of 21 CFR 610.70, you may report the status to FDA as a "PMC Submission – Correspondence/Status Update." The status report for each commitment should include:

- The original schedule for the commitment, and
- The status of the commitment (i.e., pending, ongoing, delayed, terminated, or submitted).

When you have fulfilled your commitment, submit your final report as PMC Submission – Final Report or Supplement Contains Postmarketing Commitment – Final Report.

If you have any questions, please contact LCDR Edward Wolfgang, at (301)-827-3070.

Sincerely yours,

Florence Hart MD sept for NB

Norman W. Baylor, Ph.D.
Director
Office of Vaccines
Research and Review
Center for Biologics
Evaluation and Research

EXHIBIT F

US005877298A

United States Patent [19]

Fahim et al.

[11] Patent Number: 5,877,298

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[54] ACELLULAR PERTUSSIS VACCINES AND METHODS OF PREPARING THEREOF

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[58] **Field of Search** 424/241.1, 242.1, 424/253.1, 254.1, 234.1, 240.1; 435/7.2, 7.3, 822; 530/403, 412, 413, 414, 415, 416, 417, 418, 420, 421

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[57] **ABSTRACT**

A fimbrial agglutinogen preparation is prepared from a bordetella strain, particularly a *B. pertussis* strain, by a multiple step procedure involving extraction of the fimbrial agglutinogens from cell paste and concentrating and purifying the extracted material. The fimbrial agglutinogen preparation may be used to prepare acellular pertussis vaccines with other pertussis antigens, including pertussis toxin or toxoid thereof, the 69 kDa protein and filamentous hemagglutinin and other Bordetella antigens.

12 Claims, 1 Drawing Sheet

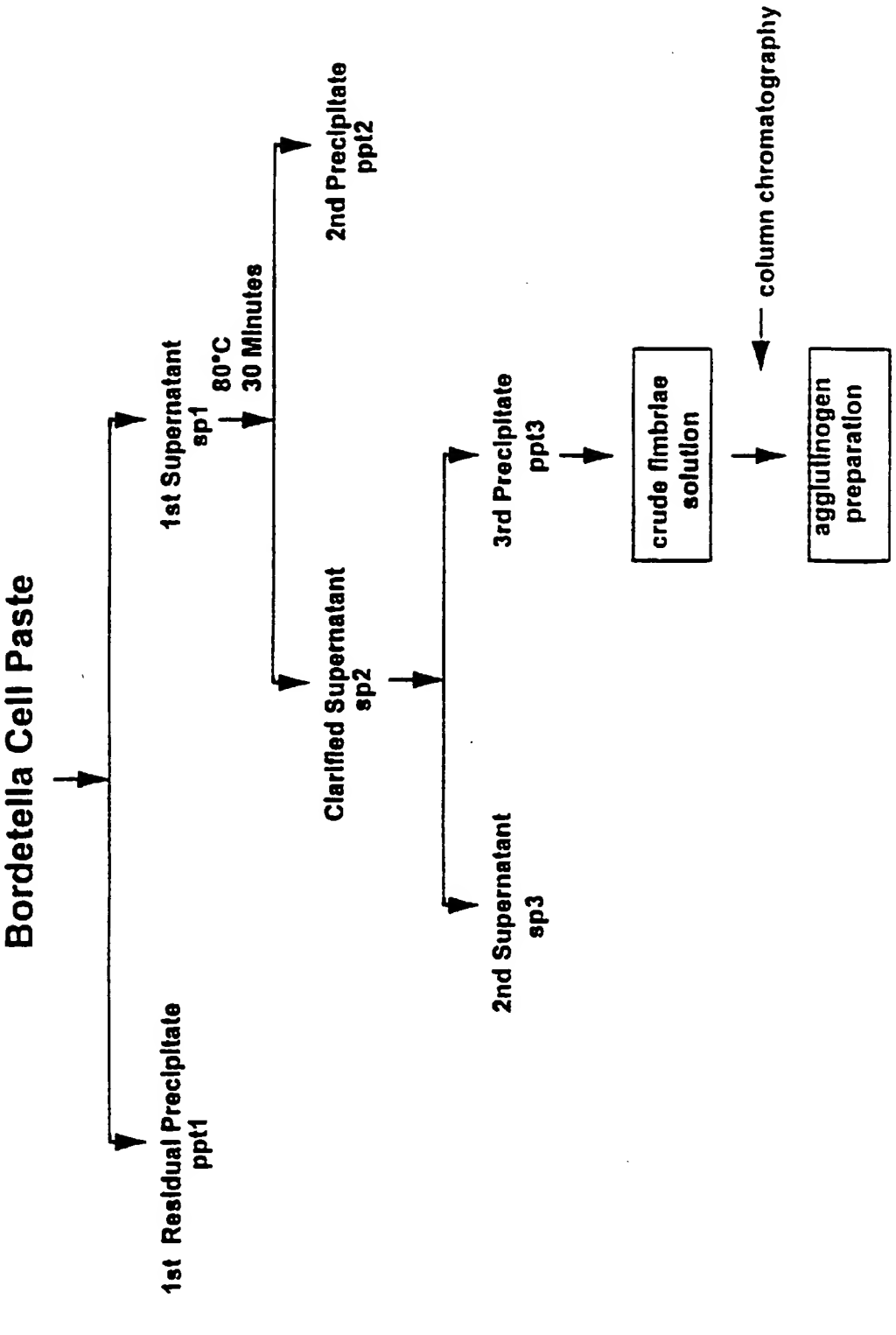
Bordetella Cell Paste
1st Residual Precipitate
ppt1
1st Supernatant
sp1
80°C
30 Minutes
2nd Precipitate
ppt2
Clarified Supernatant
sp2
2nd Supernatant
sp3
3rd Precipitate
ppt3
crude fimbriae
solution
column chromatography
agglutinogen
preparation

Figure 1

ACELLULAR PERTUSSIS VACCINES AND METHODS OF PREPARING THEREOF

FIELD OF INVENTION

The present invention relates to acellular pertussis vaccines, components thereof, and their preparation.

BACKGROUND TO THE INVENTION

Whooping cough or pertussis is a severe, highly contagious upper respiratory tract infection caused by *Bordetella pertussis*. The World Health Organization estimates that there are 60 million cases of pertussis per year and 0.5 to 1 million associated deaths (ref. 1. Throughout this specification, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately following the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). In unvaccinated populations, a pertussis incidence rate as high as 80% has been observed in children under 5 years old (ref. 2). Although pertussis is generally considered to be a childhood disease, there is increasing evidence of clinical and asymptomatic disease in adolescents and adults (refs. 3, 4 and 5).

The introduction of whole-cell vaccines composed of chemically- and heat-inactivated *B. pertussis* organisms in the 1940's was responsible for a dramatic reduction in the incidence of whooping cough caused by *B. pertussis*. The efficacy rates for whole-cell vaccines have been estimated at up to 95% depending on case definition (ref. 6). While infection with *B. pertussis* confers life-long immunity, there is increasing evidence for waning protection after immunization with whole-cell vaccines (ref. 3). Several reports citing a relationship between whole-cell pertussis vaccination, reactogenicity and serious side-effects led to a decline in vaccine acceptance and consequent renewed epidemics (ref. 7). More recently defined component pertussis vaccines have been developed.

Antigens for Defined Pertussis Vaccines

Various acellular pertussis vaccines have been developed and include the *Bordetella pertussis* antigens, Pertussis Toxin (PT), Filamentous hemagglutinin (FHA), the 69 kDa outer membrane protein (pertactin) and fimbrial agglutinogens (see Table 1 below. The Tables appear at the end of the specification).

Pertussis Toxin

Pertussis toxin is an exotoxin which is a member of the A/B family of bacterial toxins with ADP-ribosyltransferase activity (ref. 8). The A-moiety of these toxins exhibit the ADP-ribosyltransferase activity and the B portion mediates binding of the toxin to host cell receptors and the translocation of A to its site of action. PT also facilitates the adherence of *B. pertussis* to ciliated epithelial cells (ref. 9) and also plays a role in the invasion of macrophages by *B. pertussis* (ref. 10).

All acellular pertussis vaccines have included PT, which has been proposed as a major virulence factor and protective antigen (ref. 11, 12). Natural infection with *B. pertussis* generates both humoral and cell-mediated responses to PT (refs. 13 to 17). Infants have transplacentally-derived anti-PT antibodies (refs. 16, 18) and human colostrum containing anti-PT antibodies was effective in the passive protection of

mice against aerosol infection (ref. 19). A cell-mediated immune (CMI) response to PT subunits has been demonstrated after immunization with an acellular vaccine (ref. 20) and a CMI response to PT was generated after whole-cell vaccination (ref. 13). Chemically-inactivated PT in whole-cell or component vaccines is protective in animal models and in humans (ref. 21) Furthermore, monoclonal antibodies specific for subunit S1 protect against *B. pertussis* infection (refs. 22 and 23).

The main pathophysiological effects of PT are due to its ADP-ribosyltransferase activity. PT catalyses the transfer of ADP-ribose from NAD to the G_i guanine nucleotide-binding protein, thus disrupting the cellular adenylate cyclase regulatory system (ref. 24). PT also prevents the migration of macrophages and lymphocytes to sites of inflammation and interferes with the neutrophil-mediated phagocytosis and killing of bacteria (ref. 25). A number of in vitro and in vivo assays have been used to asses the enzymatic activity of S1 and/or PT, including the ADP-ribosylation of bovine transducin (ref. 26), the Chinese hamster ovary (CHO) cell clustering assay (ref. 27), histamine sensitization (ref. 28), leukocytosis, and NAD glycohydrolase. When exposed to PT, CHO cells develop a characteristic clustered morphology. This phenomenon is dependent upon the binding of PT, and subsequent translocation and ADP-ribosyltransferase activity of S1 and thus the CHO cell clustering assay is widely used to test the integrity and toxicity of PT holotoxins.

Filamentous Hemagglutinin

Filamentous hemagglutinin is a large (220 kDa) non-toxic polypeptide which mediates attachment of *B. pertussis* to ciliated cells of the upper respiratory tract during bacterial colonization (refs. 9, 29). Natural infection induces anti-FHA antibodies and cell mediated immunity (refs. 13, 15, 17, 30 and 31). Anti-FHA antibodies are found in human colostrum and are also transmitted transplacentally (refs. 17, 18 and 19). Vaccination with whole-cell or acellular pertussis vaccines generates anti-FHA antibodies and acellular vaccines containing FHA also induce a CMI response to FHA (refs. 20, 32). FHA is a protective antigen in a mouse respiratory challenge model after active or passive immunization (refs. 33, 34). However, alone FHA does not protect in the mouse intracerebral challenge potency assay. (ref. 28).

69 kDa Outer Membrane Protein (Pertactin)

The 69 kDa protein is an outer membrane protein which was originally identified from *B. bronchiseptica* (ref. 35). It was shown to be a protective antigen against *B. bronchiseptica* and was subsequently identified in both *B. pertussis* and *B. parapertussis*. The 69 kDa protein binds directly to eukaryotic cells (ref. 36) and natural infection with *B. pertussis* induces an anti-P.69 humoral response (ref. 14) and P.69 also induces a cell-mediated immune response (ref. 17, 37, 38). Vaccination with whole-cell or acellular vaccines induces anti-P.69 antibodies (refs. 32, 39) and acellular vaccines induce P.69 CMI (ref. 39). Pertactin protects mice against aerosol challenge with *B. pertussis* (ref. 40) and in combination with FHA, protects in the intracerebral challenge test against *B. pertussis* (ref. 41). Passive transfer of polyclonal or monoclonal anti-P.69 antibodies also protects mice against aerosol challenge (ref. 42).

Agglutinogens

Serotypes of *B. pertussis* are defined by their agglutinating fimbriae. The WHO recommends that whole-cell vac-

cines include types 1, 2 and 3 agglutinogens (Aggs) since they are not cross-protective (ref. 43). Agg 1 is non-fimbrial and is found on all *B. pertussis* strains while the serotype 2 and 3 Aggs are fimbrial. Natural infection or immunization with whole-cell or acellular vaccines induces anti-Agg antibodies (refs. 15, 32). A specific cell-mediated immune response can be generated in mice by Agg 2 and Agg 3 after aerosol infection (ref. 17). Aggs 2 and 3 are protective in mice against respiratory challenge and human colostrum containing anti-agglutinogens will also protect in this assay (refs. 19, 44, 45).

Acellular Vaccines

The first acellular vaccine developed was the two-component PT+FHA vaccine (JNIH 6) of Sato et al. (ref. 46). This vaccine was prepared by co-purification of PT and FHA antigens from the culture supernatant of *B. pertussis* strain Tohama, followed by formalin toxoiding. Acellular vaccines from various manufacturers and of various compositions have been used successfully to immunize Japanese children against whopping cough since 1981 resulting in a dramatic decrease in incidence of disease (ref. 47). The JNIH 6 vaccine and a mono-component PT toxoid vaccine (JNIH 7) were tested in a large clinical trial in Sweden in 1986. Initial results indicated lower efficacy the reported efficacy of a whole-cell vaccine, but follow-up studies have shown it to be more effective against milder disease diagnosed by serological methods (refs. 48, 49, 50, 51). However, there was evidence for reversion to toxicity of formalin-inactivated PT in these vaccines. These vaccines were also found to protect against disease rather than infection.

Thus, current commercially-available acellular pertussis vaccines may not contain appropriate formulations of appropriate antigens in appropriate immunogenic forms to achieve a desired level of efficacy in a pertussis-susceptible human population.

A number of new acellular pertussis vaccines are currently being assessed which include combinations of PT, FHA, P.69, and/or agglutinogens and these are listed in Table 1. Several techniques of chemical detoxication have been used for PT including inactivation with formalin (ref. 46), glutaraldehyde (ref. 52), hydrogen peroxide (ref. 53), and tetranitromethane (ref. 54).

SUMMARY OF THE INVENTION

The present invention is directed towards acellular pertussis vaccine preparations, components thereof and methods of preparation of such vaccines and their components.

In accordance with one aspect of the invention there is provided a process for preparing an agglutinogen preparation from a Bordetella strain, comprising the steps of:

(a) providing a cell paste of the Bordetella strain;

(b) selectively extracting fimbrial agglutinogens from the cell paste to produce a first supernatant containing the agglutinogens and a first residual precipitate;

(c) separating the first supernatant from the first residual precipitate;

(d) incubating the first supernatant at a temperature and for a time to produce a clarified supernatant containing fimbrial agglutinogens and a second precipitate containing non-agglutinogen contaminants;

(e) concentrating the clarified supernatant to produce a crude fimbrial agglutinogen containing solution; and

(f) purifying agglutinogens from the crude fimbriae solution to produce the agglutinogen preparation.

The Bordetella strain may be *B. pertussis*. The first supernatant may be incubated at a temperature of about 50° C. to about 100° C., including about 75° C. to about 85° C., preferably about 80° C. The time of incubation may be about 10 minutes to about 60 minutes, preferably about 30 minutes. The fimbrial agglutinogens may be selectively extracted from the cell paste by dispersing the cell paste in a buffer comprising about 1M to about 6M urea. In a particular embodiment, the first supernatant is concentrated before incubating at the time and temperature to produce the clarified supernatant.

The clarified supernatant may be concentrated by any convenient means including precipitating fimbrial agglutinogens from the clarified supernatant, separating the precipitated fimbrial agglutinogens from the resulting supernatant, and solubilizing the precipitated fimbrial agglutinogens. The precipitation may be effected by the addition of a polyethylene glycol, such as a polyethylene glycol of molecular weight of about 8000, to the clarified supernatant. The concentration of polyethylene glycol employed in such precipitation may be about 3% to about 5%, preferably about 4.3 to about 4.7%, to effect precipitation of said fimbrial agglutinogens from the clarified supernatant.

The fimbrial agglutinogens may be purified from the crude fimbriae solution by column chromatography and the column chromatography may include gel filtration, such as by the use of Sephadex 6B and/or PEI silica column chromatography. In a particular aspect of the invention, the agglutinogens are provided as a sterile agglutinogen preparation sterilized by, for example, sterile-filtration of the run-through from the column chromatography purification. In a particular embodiment, the sterile fimbrial agglutinogen preparation is adsorbed onto a mineral salt adjuvant, which may be alum.

In a particular aspect of the invention, there is provided a fimbrial agglutinogen preparation from a Bordetella strain comprising fimbrial agglutinogen 2 (Agg 2) and fimbrial agglutinogen 3 (Agg 3) substantially free from agglutinogen 1. Since agglutinogen 1 is reported to be the lipooligosaccharide (LOS) of *B. pertussis* which is reactogenic, the provision of a fimbrial agglutinogen substantially free of LOS, therefore, reduces the reactogenicity due thereto. The weight ratio of Agg 2 to Agg 3 may be from about 1.5:1 to about 2:1 in such fimbrial agglutinogen preparation. In a particular embodiment of the present invention, there is provided a fimbrial agglutinogen preparation prepared by the method as provided herein.

In a further aspect of the invention, there is provided an immunogenic composition comprising the fimbrial agglutinogen preparation as provided herein. The immunogenic composition may be formulated as a vaccine for in vivo use for protecting a host immunized therewith from disease caused by Bordetella and may comprise at least one other Bordetella antigen. The at least one other Bordetella antigen may be filamentous haemagglutinin, the 69 kDa outer membrane protein adenylate cyclase, Bordetella lipooligosaccharide, outer membrane proteins and pertussis toxin or a toxoid thereof, including genetically detoxified analogs thereof.

The immunogenic composition may comprise pertussis toxoid, filamentous haemagglutinin and agglutinogens of *B. pertussis* at a weight ratio of about 2:1:1 as provided, for example, by about 10 μg of pertussis toxoid, about 5 μg of filamentous haemagglutinin and about 5 μg of agglutinogens in a single human dose. In an alternative embodiment, the immunogenic composition may comprise pertussis toxoid, filamentous haemagglutinin, the 69 kDa protein and fila-

mentous agglutinogens of Bordetella at a weight ratio of about 10:5:5:3 as provided by about 10 μg of pertussis toxoid, about 5 μg of filamentous haemagglutinin, about 5 μg of 69 kDa protein and about 3 μg of fimbrial agglutinogens in a single human dose. In a further particular embodiment, the immunogenic composition may comprise pertussis toxoid, filamentous haemagglutinin, 69 kDa protein and fimbrial agglutinogens of *B. pertussis* in a weight ratio of about 20:20:5:3 and such ratio may be provided by about 20 μg of pertussis toxoid, about 20 μg of filamentous haemagglutinin, about 5 μg of 69 kDa protein and about 3 μg of fimbrial agglutinogens in a single human dose. In a yet further particular embodiment, the immunogenic composition may comprise pertussis toxoid filamentous haemagglutinin, 69 kDa protein and fimbrial agglutinogens in a weight ratio of about 20:10:10:6 and such ratio may be provided by about 20 μg of pertussis toxoid, about 10 μg of filamentous haemagglutinin, about 10 μg of 69 kDa protein and about 6 μg of fimbrial agglutinogens in a single human dose.

In a such particular embodiments, the immunogenic compositions provide for an immune response profile to each of the antigens contained therein and the response profile is substantially equivalent to that produced by a whole cell pertussis vaccine.

In a further aspect of the invention, the immunogenic composition as provided herein may comprise at least one non-Bordetella immunogen. Such non-Bordetella immunogen may be diphtheria toxoid, tetanus toxoid, capsular polysaccharide of Haemophilus, outer membrane protein of Haemophilus, hepatitis B surface antigen, polio, mumps, measles and/or rubella. In a particularly desirable embodiment of the invention, there is provided an immunogenic composition comprising pertussis toxoid, filamentous haemagglutinin, 69 kDa protein and fimbrial agglutinogens of *B. pertussis* in a weight ratio of about 20:20:5:3 and further comprising diphtheria toxoid in the amount of, for example, about 15 Lfs and tetanus toxoid in the amount of about 5 Lfs in a single human dose.

The immunogenic compositions as provided herein may further comprise an adjuvant and such adjuvant may be aluminum phosphate, aluminum hydroxide, Quil A, QS21, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octodecyl ester of an amino acid or a lipoprotein.

In a further aspect of the invention, there is provided a method of immunizing a host against disease caused by Bordetella, comprising administering to the host, which may be human, an immunoeffective amount of the immunogenic composition as provided herein.

Advantages of the present invention include a simple process for the preparation of immunogenic agglutinogen preparations suitable for inclusion in acellular pertussis vaccines to increase the efficacy of such vaccines.

Agglutinogen preparations provided by the present invention have utility in the formulation of acellular multicomponent vaccines for protecting a host immunized therewith from disease caused by Bordetella including *B. pertussis*. In particular, the immunogenic compositions containing agglutinogen preparations as provided herein have been selected by the Food and Drug Administration of the United States Government for evaluation in a double-blind, human efficacy clinical trial, thereby establishing a sufficient basis to those especially skilled in the art that the compositions will be effective to some degree in preventing the stated disease (pertussis). This trial is ongoing as of the date of filing of this U.S. patent application. The subject of that trial (being a vaccine as provided herein) has met the burden of being reasonably predictive of utility.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be further understood from the following detailed description and Examples with reference to the accompanying drawing in which:

FIG. 1 is a schematic flow sheet of a procedure for the isolation of an agglutinogen preparation from a Bordetella strain in accordance with one aspect of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present invention provides novel techniques which can be employed for preparing agglutinogen preparations from a Bordetella strain. Referring to FIG. 1, there is illustrated a flow sheet of a method for preparing an agglutinogen preparation from a Bordetella strain. As seen in FIG. 1, a Bordetella cell paste containing the agglutinogens, such as *B. pertussis* cell paste, is extracted with, for example, a urea-containing buffer, such as 10 mM potassium phosphate, 150 mM Nacl and 4M urea, to selectively extract the agglutinogens from the cell paste to produce a first supernatant (sp1) containing agglutinogens and a first residual precipitate (ppt1). The first supernatant (sp1) is separated from the first residual precipitate (ppt1) such as by centrifugation. The residual precipitate (ppt1) is discarded. The clarified supernatant (sp1) then may be concentrated and diafiltered against, for example, 10 mM potassium phosphate/150 mM NaCl/0.1% Triton X-100 using, for example, a 100 to 300 kDa NMWL membrane filter.

The first supernatant then is incubated at a temperature and for a time to produce a clarified supernatant (sp2) containing agglutinogens and a second discard precipitate (ppt2) containing non-agglutinogen contaminants. Appropriate temperatures include about 50° C. to about 100° C., including about 75° to about 85° C., and appropriate incubation times include about 1 to about 60 minutes. The clarified supernatant then is concentrated by, for example, the addition of polyethylene glycol of molecular weight about 8000 (PEG 8000) to a final concentration of about 4.5±0.2% and stirring gently for a minimum of about 30 minutes to produce a third precipitate (ppt3) which may be collected by centrifugation. The remaining supernatant sp3 is discarded.

This third precipitate (ppt3) is extracted with, for example, a buffer comprising 10 mM potassium phosphate/150 mM NaCl to provide the crude fimbrial agglutinogen-containing solution. 1M potassium phosphate may be added to the crude fimbrial solution to make it about 100 mM with respect to potassium phosphate. Alternatively, the clarified supernatant of heat-treated fimbrial agglutinogens can be purified without precipitation by gel-filtration chromatography using a gel, such as Sepharose CL6B. The fimbrial agglutinogens in the crude solution then are purified by column chromatography, such as, by passing through a PEI silica column, to produce the fimbrial agglutinogen preparation in the run-through.

This fimbrial agglutinogen containing run-through may be further concentrated and diafiltered against, for example, a buffer containing 10mM potassium phosphate/150 mM NaCl using a 100–300 kDa NMWL membrane. The agglutinogen preparation may be sterilized by filtration through a ≦0.22 μM membrane filter, to provide the final purified fimbrial agglutinogen preparation containing fimbrial agglutinogen 2 and 3.

The present invention extends to an agglutinogen preparation from a Bordetella strain comprising fimbrial agglutinogen 2 (Agg 2) and fimbrial agglutinogen 3 (Agg 3) substantially free from agglutinogen 1. The weight ratio of Agg 2 to Agg 3 may be from about 1.5:1 to about 2:1. Such fimbrial agglutinogen preparations may be produced by the method as provided herein and described in detail above. The present invention also extends to immunogenic compositions (including vaccines) comprising the fimbrial agglutinogen preparations as provided herein. Such vaccines may contain other Bordetella immunogens including filamentous haemagglutinin, the 69 kDa outer membrane protein and pertussis toxin or a toxoid thereof and non-Bordetella immunogens including diphtheria toxoid, tetanus toxoid, capsular polysaccharide of Haemophilus, outer membrane protein of Haemophilus, hepatitis B surface antigen, polio, mumps, measles and rubella.

In selected embodiments, the invention provides vaccines with the following characteristics (μg proteins are based on Kjedahl test results performed on purified concentrates), all of which may be administered by intramuscular injection:

(a) $CP_{10/5/5/3}DT$

One formulation of component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{10/5/5/3}DT$. Each 0.5 ml dose of $CP_{10/5/5/3}DT$ was formulated to contain about:

10 μg Pertussis toxoid (PT)

5 μg Filamentous hemagglutinin (FHA)

5 μg Fimbrial agglutinogens 2 and 3 (FIMB)

3 μg 69 kDa outer membrane protein

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol, as preservative

(b) $CP_{20/20/5/3}DT$

Another formulation of component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{20/20/5/3}DT$. Each 0.5 ml dose of $CP_{20/20/5/3}DT$ was formulated to contain about:

20 μg Pertussis toxoid (PT)

20 μg Filamentous hemagglutinin (FHA)

5 μg Fimbrial agglutinogens 2 and 3 (FIMB)

3 μg 69 kDa outer membrane protein

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol, as preservative

(c) $CP_{10/5/5}DT$

One formulation of component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{10/5/5}DT$. Each 0.5 mL dose of $CP_{10/5/5}$ was formulated to contain about:

10 μg Pertussis toxoid (PT)

5 μg Filamentous hemagglutinin (FHA)

5 μg Fimbrial agglutinogens 2 and 3 (FIMB)

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol as preservative

(d) $CP_{20/10/10/6}DT$

A further formulation of component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{20/10/10/6}DT$. Each 0.5 ml dose of $CP_{20/10/10/6}DT$ was formulated to contain about:

20 μg Pertussis toxoid (PT)

10 μg Filamentous hemagglutinin (FHA)

10 μg Fimbrial agglutinogens 2 and 3 (FIMB)

6 μg 69 kDa outer membrane protein (69 kDA)

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol, as preservative

The other Bordetella immunogens, pertussis toxin (including genetically detoxified analogs thereof, as described in, for example, Klein et al, U.S. Pat. No. 5,085,862 assigned to the assignee hereof and incorporated herein by reference thereto), FHA and the 69 kDa protein may be produced by a variety of methods such as described below:

Purification of PT

PT may be isolated from the culture supernatant of a *B. pertussis* strain using conventional methods. For example, the method of Sekura et al (ref. 55) may be used. PT is isolated by first absorbing culture supernatant onto a column containing the dye-ligand gel matrix, Affi-Gel Blue (Bio-Rad Laboratories, Richmond, Cailf.). PT is eluted from this column by high salt, such as, 0.75M magnesium chloride and, after removing the salt, is passed through a column of fetuin-Sepharose affinity matrix composed of fetuin linked to cyanogen bromide-activated Sepharose. PT is eluted from the fetuin column using 4M magnesium salt.

Alternatively, the method of Irons et al (ref. 56) may be used. Culture supernatant is absorbed onto a CNBr-activated Sepharose 4B column to which haptoglobin is first covalently bound. The PT binds to the absorbent at pH 6.5 and is eluted from the column using 0.1M Tris/0.5M NaCl buffer by a stepwise change to pH 10.

Alternatively, the method described in U.S. Pat. No. 4,705,686 granted to Scott et al on Nov. 10, 1987 and incorporated herein by reference thereto may be used. In this method culture supernatants or cellular extracts of *B. pertussis* are passed through a column of an anion exchange resin of sufficient capacity to adsorb endotoxin but permit Bordetella antigens to flow through or otherwise be separated from the endotoxin.

Alternatively, PT may be purified by using perlite chromatography, as described in EP Patent No. 336 736, assigned to the assignee thereof and incorporated herein by reference thereto.

Detoxification of PT

PT is detoxified to remove undesired activities which could cause side reactions of the final vaccine. Any of a variety of conventional chemical detoxification methods can be used, such as treatment with formaldehyde, hydrogen peroxide, tetranitro-methane, or glutaraldehyde.

For example, PT can be detoxified with glutaraldehyde using a modification of the procedure described in Munoz et al (ref. 57). In this detoxification process purified PT is incubated in a solution containing 0.01M phosphate buffered saline. The solution is made 0.05% with glutaraldehyde and the mixture is incubated at room temperature for two hours, and then made 0.02M with L-lysine. The mixture is further incubated for two hours at room temperature and then dialyzed for two days against 0.01M PBS. In a particular embodiment, the detoxification process of EP Patent No. 336

736 may be used. Briefly PT may be detoxified with glutaraldehyde as follows:

Purified PT in 75 mM potassium phosphate at pH 8.0 containing 0.22M sodium chloride is diluted with an equal volume of glycerol to protein concentrations of approximately 50 to 400 μg/ml. The solution is heated to 37° C. and detoxified by the addition of glutaraldehyde to a final concentration of 0.5% (w/v). The mixture is kept at 37° C. for 4 hrs and then aspartic acid (1.5M) is added to a final concentration of 0.25M. The mixture is incubated at room temperature for 1 hour and then diafiltered with 10 volumes of 10 mM potassium phosphate at pH 8.0 containing 0.15M sodium chloride and 5% glycerol to reduce the glycerol and to remove the glutaraldehyde. The PT toxoid is sterile-filtered through a 0.2 μM membrane.

If recombinant techniques are used to prepare a PT mutant molecule which shows no or little toxicity, chemical detoxification is not necessary.

Purification of FHA

FHA may be purified from the culture supernatant essentially as described by Cowell et al (ref. 58). Growth promoters, such as methylated beta-cyclodextrins, may be used to increase the yield of FHA in culture supernatants. The culture supernatant is applied to a hydroxylapatite column. FHA is adsorbed onto the column, but PT is not. The column is extensively washed with Triton X-100 to remove endotoxin. FHA is then eluted using 0.5M NaCl in 0.1M sodium phosphate and, if needed, passed through a fetuin-Sepharose column to remove residual PT. Additional purification can involve passage though a Sepharose CL-6B column.

Alternatively, FHA may be purified using monoclonal antibodies to the antigen, where the antibodies are affixed to a CNBr-activated affinity column (ref. 59).

Alternatively, FHA may be purified by using perlite chromatography as described in the above-mentioned EP 336 736.

Purification of 69 kDa Outer Membrane Protein (pertactin)

The 69 kDa outer membrane protein may be recovered from bacterial cells by first inactivating the cells with a bacteriostatic agent, such as thimerosal, as described in published EP 484 621. The inactivated cells are suspended in an aqueous medium, such as PBS (pH 7 to 8) and subjected to repeated extraction at elevated temperature (45° to 60° C.) with subsequent cooling to room temperature or 4° C. The extractions release the 69K protein from the cells. The material containing the 69K protein is collected by precipitation and passed through an Affi-gel Blue column. The 69K protein is eluted with a high concentration of salt, such as 0.5M magnesium chloride. After dialysis, it is passed through a chromatofocusing support.

Alternatively, the 69 kDa protein may be purified from the culture supernatant of a *B. pertussis* culture, as described in published PCT Application WO 91/15505, in the name of the assignee hereof and incorporated herein by reference thereto.

Other appropriate methods of purification of the 69 kDa outer membrane protein from *B. pertussis* are described in U.S. Pat. No. 5,276,142, granted to Gotto et al on Jan. 4, 1984 and in U.S. Pat. No. 5,101,014, granted to Burns on Mar. 31, 1992.

A number of clinical trials were performed in humans as described herein to establish the safety, non-reactogenicity and utility of component vaccines containing fimbrial agglutinogens prepared as described herein, for protection against pertussis. In particular, immune responses to each of the antigens contained in the vaccines (as shown, for example, in Table 3 below) were obtained. In particular, the profile of immune response obtained was substantially the same as that obtained following immunization with whole-cell obtained following immunization with whole-cell pertussis vaccines which are reported to be highly efficacious against pertussis.

Vaccine Preparation and Use

Thus, immunogenic compositions, suitable to be used as vaccines, may be prepared from the Bordetella immunogens as disclosed herein. The vaccine elicits an immune response in a subject which produces antibodies that may be opsonizing or bactericidal. Should the vaccinated subject be challenged by *B. pertussis*, such antibodies bind to and inactivate the bacteria. Furthermore, opsonizing or bactericidal antibodies may also provide protection by alternative mechanisms.

Immunogenic compositions including vaccines may be prepared as injectibles, as liquid solutions or emulsions. The Bordetella immunogens may be mixed with pharmaceutically acceptable excipients which are compatible with the immunogens. Such excipients may include water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously or intramuscularly. The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, immunogenic and protective. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the immune system of the individual to synthesize antibodies, and, if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the immunogens. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host.

The concentration of the immunogens in an immunogenic composition according to the invention is in general about 1 to about 95%. A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as 0.005 to 0.5 percent solution in phosphate buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system.

Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established and, more recently, a HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are often emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and Pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants include:

(1) lack of toxicity;

(2) ability to stimulate a long-lasting immune response;

(3) simplicity of manufacture and stability in long-term storage;

(4) ability to elicit both CMI and HIR to antigens administered by various routes;

(5) synergy with other adjuvants;

(6) capability of selectively interacting with populations of antigen presenting cells (APC):

(7) ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

U.S. Pat. No. 4,855,283 granted to Lockhoff et al on Aug. 8, 1989 which is incorporated herein by reference thereto teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. (U.S. Pat. No. 4,855,283 and ref. 60) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycosphingolipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Pat. No. 4,258,029 granted to Moloney, assigned to the assignee hereof and incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al. (ref. 61), reported that octodecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for the purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Methods of protein biochemistry, fermentation and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example describes the growth of *Bordetella pertussis*.

Master Seed:

Master seed cultures of a *Bordetella pertussis* strain were held as freeze-dried seed lots, at 2° C. to 8° C.

Working Seed:

The freeze-dried culture was recovered in Hornibrook medium and used to seed Bordet-Gengou Agar (BGA) plates. Hornibrook medium has the following composition:

Component	for 1 liter
Casein hydrolysate (charcoal treated)	10.0 g
Nicotinic acid	0.001 g
Calcium chloride	0.002 g
Sodium chloride	5.0 g
Magnesium chloride hexahydrate	0.025 g
Potassium chloride	0.200 g
Potassium phosphate dibasic	0.250 g
Starch	1.0 g
Distilled water	to 1.0 liter

The pH is adjusted to 6.9±0.1 with 1% sodium carbonate solution. The medium is dispensed into tubes and sterilized by steaming in the autoclave for 20 minutes and autoclaving for 20 minutes at 121° C. to 124° C. The seed was subcultured twice, firstly on BGA plates then on Component Pertussis Agar (CPA). Component Pertussis Agar (CPA) has the following composition:

NaCl	2.5 g/L
KH_2PO_4	0.5 g/L
KCl	0.2 g/L
$MgCl_2(H_20)_6$	0.1 g/L
Tris base	1.5 g/L
Casamino acids	10.0 g/L
NaHGlutamate	10.0 g/L
Conc. HCl	to pH 7.2
Agar	15.0 g/L
Growth factors (CPGF)	10.0 mL/L

Component Pertussis Growth Factors (CPGF)—100X have the following composition:

L-cysteine HCl	4.0 g/L
Niacin	0.4 g/L
Ascorbic acid	40.0 g/L
Glutathione, reduced	15.0 g/L
$Fe_2SO_4.(H_2O)_7$	1.0 g/L
Dimethyl-β-cyclodextrin	100 g/L
$CaCl_2(H_2O)_2$	2.0 g/L

The final culture was suspended in Pertussis Seed Suspension Buffer (CPSB), dispensed into 2 to 4 ml aliquots and stored frozen at −60° C. to −85° C. Pertussis Seed Suspension Buffer (PSSB) has the following composition:

Casamino acids	10.0 g/L
Tris base	1.5 g/L
Anhydrous glycerol	100 mL/L
Conc. HCl	to pH 7.2

These glycerol suspensions provided the starting material for the preparation of the working seed.

Cultivation Process:

Propagation of the working seed was conducted in Component Pertussis Agar Roux bottles for 4 to 7 days at 34° C. to 38° C. Following this cultivation, cells were washed off agar with Component Pertussis Broth (CPB). Samples were observed by Gram stain, for culture purity and opacity.

Cells were transferred to 4 liter conical flasks containing CPB and incubated at 34° C. to 38° C. for 20 to 26 hours with shaking. Samples were observed by Gram stain and culture purity. Flasks were pooled and the suspension was used to seed two fermenters containing CPB (10 liter volume starting at OD_{600} 0.1–0.4). The seed was grown to a final OD_{600} of 5.0 to 10.0. Samples were tested by Gram strain, for culture purity, by antigen specific ELISAs and for sterility.

Example 2

This Example describes the purification of antigens from the *Bordetella pertussis* cell culture.

Production of Broth and Cell Concentrates:

Bacterial suspension was grown in two production fermenters, at 34° C. to 37° C. for 35 to 50 hours. The fermenters were sampled for media sterility testing. The suspension was fed to a continuous-flow disk-stack centrifuge (12,000×g) to separate cells from the broth. Cells were collected to await extraction of fimbriae component. The clarified liquor was passed through ≦0.22 μm membrane filter. The filtered liquor was concentrated by ultra filtration using a 10 to 30 kDa nominal molecular weight limit (NMWL) membrane. The concentrate was stored to await separation and purification of the Pertussis Toxin (PT), Filamentous hemagglutinin (FHA) and 69 kDa (pertactin) components.

Separation of the Broth Components:

The broth components (69 kDa, PT and FHA) were separated and purified by perlite chromatography and selective elution steps, essentially as described in EP Patent No. 336 736 and applicants published PCT Application No. WO 91/15505, described above. The specific purification operations effected are described below.

Pertussis Toxin (PT):

The perlite column was washed with 50 mM Tris, 50 mM Tris/0.5% Triton X-100 and 50 mM Tris buffers. The PT fraction was eluted from the perlite column with 50 mM Tris/0.12M NaCl buffer.

The PT fraction from the perlite chromatography was loaded onto a hydroxylapatite column and then washed with 30 mM potassium phosphate buffer. PT was eluted with 75 mM potassium phosphate/225 mM NaCl buffer. The column was washed with 200 mM potassium phosphate/0.6M NaCl to obtain the FHA fraction which was discarded. Glycerol was added to the purified PT to 50% and the mixture was stored at 2° C. to 8° C. until detoxification, within one week.

Filamentous Hemagglutinin (FHA):

The FHA fraction was eluted from the perlite column with 50 mM Tris/0.6M NaCl. Filamentous haemagglutinin was purified by chromatography over hydroxylapatite. The FHA fraction from the perlite column was loaded onto a hydroxylapatite column then washed with 30 mM potassium phosphate containing 0.5% Triton X-100, followed by 30 mM potassium phosphate buffer. The PT fraction was eluted with 85 mM potassium phosphate buffer and discarded. The FHA fraction was then eluted with 200 mM potassium phosphate/0.6M NaCl and stored at 2° C. to 8° C. until detoxification within one week.

69 kDa (pertactin):

The broth concentrate was diluted with water for injection (WFI) to achieve a conductivity of 3 to 4 mS/cm and loaded onto a perlite column at a loading of 0.5 to 3.5 mg protein per ml perlite. The run-through (69 kDa Component Fraction) was concentrated by ultrafiltration using a 10 to 30 kDa NMWL membrane. Ammonium sulphate was added to the run-through concentrate to 35%±3% (w/v) and the resulting mixture stored at 2° C. to 8° C. for 4±2 days or centrifuged (7,000×g) immediately. Excess supernatant was decanted and the precipitate collected by centrifugation (7,000×g). The 69 kDa pellet was either stored frozen at −20° C. to −30° C. or dissolved in Tris or phosphate buffer and used immediately.

The 69 kDa outer membrane protein obtained by the 35% (w/v) ammonium sulphate precipitation of concentrated perlite run-through was used for the purification. Ammonium sulphate (100±5 g per liter) was added to the 69 kDa fraction and the mixture stirred for at least 2 hours at 2° C. to 8° C. The mixture was centrifuged (7,000×g) to recover the supernatant. Ammonium sulphate (100 to 150 g per liter) was added to the supernatant and the mixture stirred for at least 2 hours at 2° C. to 8° C. The mixture was centrifuged (7,000×g) to recover the pellet, which was dissolved in 10 mM Tris, HCl, pH 8. The ionic strength of the solution was adjusted to the equivalent of 10 mM Tris HCl (pH 8), containing 15 mM ammonium sulphate.

The 69 kDa protein was applied to a hydroxylapatite column connected in tandem with a Q-Sepharose column. The 69 kDa protein was collected in the run-through, was flushed from the columns with 10 mM Tris, HCl (pH 8), containing 15 mM ammonium sulphate and pooled with 69 kDa protein in the run-through. The 69 kDa protein pool was diafiltered with 6 to 10 volumes of 10 mM potassium phosphate (pH 8), containing 0.15M NaCl on a 100 to 300

kDa NMWL membrane. The ultra filtrate was collected and the 69 kDa protein in the ultra filtrate concentrated.

The 69 kDa protein was solvent exchanged into 10 mM Tris HCl (pH8), and adsorbed onto Q-Sepharose, washed with 10 mM Tris HCl (pH 8)/5 mM ammonium sulphate. The 69 kDa protein was eluted with 50 mM potassium phosphate (pH 8). The 69 kDa protein was diafiltered with 6 to 10 volumes of 10 mM potassium phosphate (pH 8) containing 0.15M NaCl on a 10 to 30 kDa NMWL membrane. The 69 kDa protein was sterile filtered through a ≦0.22 μm filter. This sterile bulk was stored at 2° C. to 8° C. and adsorption was performed within three months.

Fimbrial Agglutinogens:

The agglutinogens were purified from the cell paste following separation from the broth. The cell paste was diluted to a 0.05 volume fraction of cells in a buffer containing 10 mM potassium phosphate, 150 mM NaCl and 4M urea and was mixed for 30 minutes. The cell lysate was clarified by centrifugation (12,000×g) then concentrated and diafiltered against 10 mM potassium phosphate/150 mM NaCl/0.1% Triton X-100 using a 100 to 300 kDa NMWL membrane filter.

The concentrate was heat treated at 80° C. for 30 min then reclarified by centrifugation (9,000×g). PEG 8000 was added to the clarified supernatant to a final concentration of 4.5%±0.2% and stirred gently for a minimum of 30 minutes. The resulting precipitate was collected by centrifugation (17,000×g) and the pellet extracted with 10 mM potassium phosphate/150 mM NaCl buffer to provide a crude fimbrial agglutinogen solution. The fimbrial agglutinogens were purified by passage over PEI silica. The crude solution was made 100 mM with respect to potassium phosphate using 1M potassium phosphate buffer and passed through the PEI silica column.

The run-through from the columns was concentrated and diafiltered against 10 mM potassium phosphate/150 mM NaCl buffer using a 100 to 300 kDa NMWL membrane filter. This sterile bulk is stored at 2° C. to 8° C. and adsorption performed within three months. The fimbrial agglutinogen preparation contained fimbrial Agg 2 and fimbrial Agg 3 in a weight ratio of about 1.5 to about 2:1 and was found to be substantially free from Agg 1.

Example 3

This Example describes the toxoiding of the purified *Bordetella pertussis* antigens, PT and FHA.

PT, prepared in pure form as described in Example 2, was toxoided by adjusting the glutaraldehyde concentration in the PT solution to 0.5%±0.1% and incubating at 37° C.±3° C. for 4 hours. The reaction was stopped by adding L-aspartate to 0.21±0.02M. The mixture was then held at room temperature for 1±0.1 hours and then at 2° C. to 8° C. for 1 to 7 days.

The resulting mixture was diafiltered against 10 mM potassium phosphate/0.15M NaCl/5% glycerol buffer on a 30 kDa NMWL membrane filter and then sterilized by passage through a ≦0.22 μm membrane filter. This sterile bulk was stored at 2° C. to 8° C. and adsorption performed within three months.

The FHA fraction, prepared in pure form as described in Example 2, was toxoided by adjusting the L-lysine and formaldehyde concentration to 47±5 mM and 0.24±0.05% respectively and incubating at 35° C. to 38° C. for 6 weeks. The mixture was then diafiltered against 10 mM potassium phosphate/0.5M NaCl using a 30 kDa NMWL membrane filter and sterilized by passage through a membrane filter. This sterile bulk was stored a 2° C. to 8° C. and adsorption performed within three months.

Example 4

This Example describes the adsorption of the purified *Bordetella pertussis* antigens.

For the individual adsorption of PT, FHA, Agg and 69 kDa onto aluminum phosphate (alum), a stock solution of aluminum phosphate was prepared to a concentration of 18.75±1 mg/ml. A suitable vessel was prepared and any one of the antigens aseptically dispensed into the vessel. 2-phenoxyethanol was aseptically added to yield a final concentration of 0.6%±0.1% v/v and stirred until homogeneous. The appropriate volume of aluminum phosphate was aseptically added into the vessel. An appropriate volume of sterile distilled water was added to bring the final concentration to 3 mg aluminum phosphate/ml. Containers were sealed and labelled and allowed to stir at room temperature for 4 days. The vessel was then stored awaiting final formulation.

Example 5

This Example describes the formulation of a component pertussis vaccine combined with diphtheria and tetanus toxoids.

The *B. pertussis* antigens prepared as described in the preceding Examples were formulated with diphtheria and tetanus toxoids to provide several component pertussis (CP) vaccines.

The component pertussis (CP) components were produced from *Bordetella pertussis* grown in submerged culture as described in detail in Examples 1 to 4 above. After completion of growth, the culture broth and the bacterial cells were separated by centrifugation. Each antigen was purified individually. Pertussis toxin (PT) and Filamentous Haemagglutinin (FHA) were purified from the broth by sequential chromatography over perlite and hydroxylapatite. PT was detoxified with glutaraldehyde and any residual PT (approximately 1%) present in the FHA fraction was detoxified with formaldehyde. Fimbrial Agglutinogens (2+3) (AGG) were prepared from the bacterial cells. The cells were disrupted with urea and heat treated, and the fimbrial agglutinogens were purified by precipitation with polyethylene glycol and chromatography over polyethyleneimine silica. The 69 kDa protein (pertactin) component was isolated from the perlite chromatography step by ammonium sulphate precipitation, and purified by sequential chromatography over hydroxylapatite and Q-sepharose. All components were sterilized by filtration through a 0.22 μm membrane filter.

Diphtheria toxoid was prepared from *Corynebacterium diphtheriae* grown in submerged culture by standard methods. The production of Diphtheria Toxoid is divided into five stages, namely maintenance of the working seed, growth of *Corynebacterium diphtheriae*, harvest of Diphtheria Toxin, detoxification of Diphtheria Toxin and concentration of Diphtheria Toxoid.

Preparation of Diphtheria Toxoid

(I) Working Seed

The strain of *Corynebacterium diphtheriae* was maintained as a freeze-dried seed lot. The reconstituted seed was grown on Loeffler slopes for 18 to 24 hours at 35° C.±2° C., and then transferred to flasks of diphtheria medium. The culture was then tested for purity and Lf content. The remaining seed was used to inoculate a fermenter.

(II) Growth of *Corynebacterium diphtheriae*

The culture was incubated at 35° C.±2° C. and agitated in the fermenter. Predetermined amounts of ferrous sulphate, calcium chloride and phosphate solutions were added to the culture. The actual amounts of each solution (phosphate, ferrous sulphate, calcium chloride) were determined experimentally for each lot of medium. The levels chosen are those which gave the highest Lf content. At the end of the growth cycle (30 to 50 hours), the cultures were sampled for purity, and Lf content.

The pH was adjusted with sodium bicarbonate, and the culture inactivated with 0.4% toluene for 1 hour at a maintained temperature of 35° C.±2° C. A sterility test was then performed to confirm the absence of live *C. diphtheriae.*

(III) Harvest of Diphtheria Toxin

The toluene treated cultures from one or several fermenters were pooled into a large tank. Approximately 0.12% sodium bicarbonate, 0.25% charcoal, and 23% ammonium sulphate were added, and the pH is tested.

The mixture was stirred for about 30 minutes. Diatomaceous earth was added and the mixture is pumped into a depth filter. The filtrate is recirculated until clear, then collected, and sampled for Lf content testing. Additional ammonium sulphate was added to the filtrate to give a concentration of 40%. Diatomaceous earth was also added. This mixture was held for 3 to 4 days at 2° C. to 8° C. to allow the precipitate to settle. Precipitated toxin was collected and dissolved in 0.9% saline. The diatomaceous earth was removed by filtration and the toxin dialysed against 0.9% saline, to remove the ammonium sulphate. Dialysed toxin was pooled and sampled for Lf content and purity testing.

(IV) Detoxification of Diphtheria Toxin

Detoxification takes place immediately following dialysis. For detoxification, the toxin was diluted so that the final solution contained:

a) diphtheria toxin at 1000±10% Lf/ml.

b) 0.5% sodium bicarbonate

c) 0.5% formalin

d) 0.9% w/v L-lysine monohydrochloride

The solution is brought up to volume with saline and the pH is adjusted to 7.6±0.1.

Toxoid was filtered through cellulose diatomaceous earth filter pads and/or a membrane prefilter and 0.2 μm membrane filter into the collection vessel and incubated for 5 to 7 weeks at 34° C. A sample was withdrawn for toxicity testing.

(V) Concentration of Purified Toxoid

The toxoids were pooled, then concentrated by ultrafiltration, and collected into a suitable container. Samples were taken for Lf content and purity testing. The preservative (2-phenoxyethanol) was added to give a final concentration of 0.375% and the pH adjusted to 6.6 to 7.6.

The toxoid was sterilized by filtration through a prefilter and a 0.2 μm membrane filter (or equivalent) and collected into a container. The sterile toxoid was then sampled for irreversibility of toxoid Lf content, preservative content, purity (nitrogen content), sterility, toxicity testing. The sterile concentrated toxoid were stored at 2° C. to 8° C. until final formulation.

Preparation of Tetanus Toxoid

Tetanus toxoid (T) was prepared from *Clostridium tetani* grown in submerged culture.

The production of Tetanus Toxoid can be divided into five stages, namely maintenance of the working seed, growth of *Clostridium tetani*, harvest of Tetanus Toxin, detoxification of Tetanus Toxin and purification of Tetanus Toxoid.

(I) Working Seed

The strain of *Clostridium tetani* used in the production of tetanus toxin for the conversion to tetanus toxoid was maintained in the lyophilized form in a seed-lot. The seed was inoculated into thioglycollate medium and allowed to grow for approximately 24 hours at 35° C.±2° C. A sample was taken for culture purity testing.

(II) Growth of Clostridium tetani

The tetanus medium is dispensed into a fermenter, heat-treated and cooled. The fermenter was then seeded and the culture allowed to grow for 4 to 9 days at 34° C.±2° C. A sample was taken for culture purity, and Lf content testing.

(III) Harvest of Tetanus Toxin

The toxin was separated by filtration through cellulose diatomaceous earth pads, and the clarified toxin then filter-sterilized using membrane filters. Samples were taken for Lf content and sterility testing. The toxin was concentrated by ultrafiltration, using a pore size of 30,000 daltons.

(IV) Detoxification of Tetanus Toxin

The toxin was sampled for Lf content testing prior to detoxification. The concentrated toxin (475 to 525 Lf/ml) was detoxified by the addition of 0.5% w/v sodium bicarbonate, 0.3% v/v formalin and 0.9% w/v L-lysine monohydrochloride and brought up to volume with saline. The pH was adjusted to 7.5±0.1 and the mixture incubated at 37° C. for 20 to 30 days. Samples were taken for sterility and toxicity testing.

(V) Purification of Toxoid

The concentrated toxoid was sterilized through pre-filters, followed by 0.2 μm membrane filters. Samples were taken for sterility and Lf content testing.

The optimum concentration of ammonium sulphate was based on a fractionation "S" curve determined from samples of the toxoid. The first concentration was added to the toxoid (diluted to 1900–2100 Lf/ml). The mixture was kept for at least 1 hour at 20° C. to 25° C. and the supernatant collected and the precipitate containing the high molecular weight fraction, discarded.

A second concentration of ammonium sulphate was added to the supernatant for the second fractionation to remove the low molecular weight impurities. The mixture was kept for at least 2 hours at 20° C. to 25° C. and then could be held at 2° C. to 8° C. for a maximum of three days. The precipitate, which represents the purified toxoid, was collected by centrifugation and filtration.

Ammonium sulphate was removed from the purified toxoid by diafiltration, using Amicon (or equivalent) ultrafiltration membranes with PBS until no more ammonium sulphate could be detected in the toxoid solution. The pH was adjusted to 6.6. to 7.6, and 2-phenoxyethanol added to give a final concentration of 0.375%. The toxoid was sterilized by membrane filtration, and samples are taken for testing (irreversibility of toxoid, Lf content, pH, preservative content, purity, sterility and toxicity).

One formulation of a component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{10/5/5/3}DT$. Each 0.5 ml dose of $CP_{10/5/5/3}DT$ was formulated to contain:

10 μg Pertussis toxoid (PT)

5 μg Filamentous hemagglutinin (FHA)

5 μg Fimbrial agglutinogens 2 and 3 (FIMB)

5 μg 69 kDa outer membrane protein

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol as preservative

Another formulation of component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{10/5/5}DT$. Each 0.5 ml doses of $CP_{10/5/5}DT$ was formulated to contain:

10 μg Pertussis toxoid (PT)

5 μg Filamentous hemagglutinin (FHA)

5 μg Fimbrial agglutinogens 2 and 3 (FIMB)

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol as preservative

Another formulation of Component Pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{20/20/5/3}DT$. Each 0.5 ml dose of $CP_{20/20/5/3}DT$ was formulated to contain:

20 μg Pertussis toxoid (PT)

20 μg Filamentous hemagglutinin (FHA)

5 μg Fimbrial agglutinogens 2 and 3 (FIMB)

3 μg 69 kDa outer membrane protein

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol as preservative

A further formulation of a component pertussis vaccine combined with diphtheria and tetanus toxoids was termed $CP_{20/10/10/6}DT$. Each 0.5 ml dose of $CP_{20/10/10/6}DT$ was formulated to contain:

20 μg Pertussis toxoid (PT)

10 μg Filamentous hemagglutinin (FHA)

10 μg Fimbrial agglutinogens 2 and 3 (FIMB)

6 μg 69 kDa outer membrane protein

15 Lf Diphtheria toxoid

5 Lf Tetanus toxoid

1.5 mg Aluminum phosphate

0.6% 2-phenoxyethanol as preservative

Example 6

This Example describes the clinical assessment of Component Acellular Pertussis vaccines, produced in accordance with the invention.

(a) Studies in Adults

Studies in adults and children aged 16 to 20 months indicated the multi-component vaccines containing fimbrial agglutinogens to be safe and immunogenic (Table 2).

A Phase I clinical study was performed in 17 and 18 month old children in Calgary, Alberta with the five Component Pertussis vaccine ($CP_{10/5/5/3}DT$) and the adverse reaction reported. Thirty-three children received the vaccine and additional 35 received the same vaccine without the 69 kDa protein component.

Local reactions were rare. Systemic adverse reactions, primarily consisting of irritability were present in approximately half of study participants, regardless of which vaccine was given. Significant antibody rises were measured for anti-PT, anti-FHA, anti-fimbrial agglutinogens and anti-69 kDa IgG antibodies by enzyme immunoassay and anti-PT antibodies in the CHO cell neutralization test. No differences in antibody response were detected in children who received the four component ($CP_{10/5/5}DT$) or five component ($CP_{10/5/5/3}DT$) except in the anti-69 kDa antibody. Children who received the five component vaccine containing the 69 kDa protein had a significantly higher post-immunization anti-69 kDa antibody level.

A dose-response study was undertaken with the 4 component vaccine in Winnipeg, Manitoba, Canada. Two component vaccine formulations were used: $CP_{10/5/5/3}DT$ and $CP_{20/10/10/6}DT$. A whole-cell DPT vaccine was also included as a control.

This study was a double-blind study in 91, 17 to 18 month old infants at the time of their booster pertussis dose. Both $CP_{10/5/5/3}DT$ and $CP_{20/10/10/6}DT$ were well tolerated by these children. No differences were demonstrated in the number of children who had any local reaction, or systemic reactions after either of the component vaccines. In contrast, significantly more children who received the whole-cell vaccine had local and systemic reactions than those who received the $CP_{20/10/10/6}DT$ component vaccines.

Studies in Infants:

Phase II:

A study was conducted using the $CP_{10/5/5/3}DT$ vaccine in Calgary, Alberta and British Columbia, Canada. In this study, 432 infants received the component pertussis vaccine or the whole-cell control vaccine DPT at 2, 4 and 6 months of age. The $CP_{10/5/5/3}DT$ vaccine was well tolerated by these infants. Local reactions were less common with the component vaccine than the whole cell vaccine after each dose.

A significant antibody response to all antigens was demonstrated after vaccination with the component pertussis vaccine. Recipients of the whole-cell vaccine had a vigorous antibody response to fimbrial agglutinogens, D and T. At seven months, 82% to 89% of component vaccine recipients and 92% of whole cell vaccine recipients had a four-fold increase or greater rise in antibody titer to fimbrial agglutinogens. In contrast, antibody response to FHA was 75% to 78% in component vaccinees compared to 31% of whole-cell recipients. A four-fold increase in anti-69 kDa antibody was seen in 90% to 93% of component vaccinees and 75% of whole-cell recipients. A four-fold rise in antibody against PT by enzyme immunoassay was seen in 40% to 49% of component vaccinees and 32% of whole-cell vaccinees; a four-fold rise in PT antibody by CHO neutralization was found in 55% to 69% of component and 6% of whole-cell vaccinees. (Table 2).

Phase IIB:

The $CP_{20/20/5/3}DT$ and $CP_{10/10/5/3}DT$ vaccines were assessed in a randomized blinded study against a $D_{15}PT$ control with a lower diphtheria content of 15 Lf compared to a 25 Lf formulation of 100 infants at 2, 4 and 6 months of age. No differences in rates of adverse reactions were detected between the two components formulations; both were significantly less reactogenic than the whole-cell control. Higher antibody titers against PT by enzyme immunoassay and CHO neutralization and FHA were achieved in recipients of the $CP_{20/20/5/3}DT$ vaccine with increased antigen content. At 7 months, the anti-FHA geometric mean titer was 95.0 in $CP_{20/20/5/3}DT$ recipients, 45.2 in $CP_{10/5/5/3}DT$ recipients were only 8.9 in $D_{15}PT$ recipients. Anti-PT titers were 133.3, 58.4 and 10.4 by immunoassay and 82.4, 32.7 and 4.0 by CHO neutralization respectively (Table 2).

This study demonstrated that the Component Pertussis vaccine combined with diphtheria and tetanus toxoids adsorbed, with increased antigen content, was safe and immunogenic in infants and that the increased antigen content augmented the immune response to the prepared antigens (PT and FHA) without an increase in reactogenicity.

NIAID, PHASE II, U.S. Comparative Trial:

A phase II study was performed in the United States under the auspices of the National Institute of Allergy and Infectious Diseases (NIAID) as a prelude to a large scale efficacy trial of acellular pertussis vaccines. One component pertussis vaccine of the invention in combination with diphtheria and tetanus toxoids adsorbed ($CP_{10/5/5/3}DT$) was included in that trial along with 12 other acellular vaccines and 2 whole-cell vaccines. Safety results were reported on 137 children immunized at 2, 4 and 6 months of age with the $CP_{10/5/5/3}DT$ component vaccine. As seen in previous studies, the component vaccine was found to be safe, of low reactogenicity and to be well tolerated by vaccinees.

At 7 months, anti-PT antibody, anti-FHA antibody, anti-69 kDa antibody and anti-fimbrial agglutinogens antibody were all higher than or equivalent to levels achieved after the whole-cell vaccines (ref 71 and Table 2). A double blind study was performed in which children were randomly allocated to receive either the $CP_{20/20/5}DT$ or the $CP_{10/5/5/3}DT$ vaccine formulation. A total of 2050 infants were enrolled in the United States and Canada; 1961 infants completed the study. Both vaccine formulations were safe, of low reactogenicity and immunogenic in these infants. Immunogenicity was assessed in a subgroup of 292. An antibody rise was elicited to all antigens contained in the vaccine by both vaccine formulations. The $CP_{20/20/5/3}DT$ formulation induced higher antibody titers against FHA but not PT. The $CP_{10/5/5/3}DT$ formulation elicited higher titers against fimbriae and higher agglutinogen titers (Table 7).

A further safety and immunogenicity study was conducted in France. The study design was similar to the North American study, described above, except that vaccines were administered at 2, 3 and 4 months of age. Local and systemic reactions were generally minor. Overall the vaccine was well accepted by the French study participants using this administration regime.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides novel preparations of fimbrial agglutinogens of *Bordetella pertussis* and methods for their production. The fimbrial agglutinogens can be formulated with other Bordetella and non-Bordetella antigens to produce a number of multi-component pertussis vaccines. Such vaccines are safe, non-reactogenic and immunogenic. Modifications are possible within the scope of this invention.

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TABLE 1

Acellular Pertussis Vaccines

Vaccine	PT	Toxoiding Agent	FHA	P.69	AGG2	AGG3	Reference
AMVC	+	H_2O_2[a]	–	–	–	–	64
Mass PHL[b]	+	TMN[c]	–	–	–	–	65
Institut Mérieux	+	GI[d]	+	–	–	–	66
Smith-Kline	+	FI[e]/GI	+	–	–	–	32
	+	FI/GI	+	+	–	–	32
CAMR[f]	+	FI	+	–	+	+	67
Lederle/ Takeda	+	FI	+	+	+	–	68
Connaught	+	GI	+	–	+	+	32
	+	GI	+	+	+	+	69

[a]Hydrogen peroxide inactivated.
[b]Massachusetts Public Health Laboratories.
[c]TNM, tetranitromethane-inactivated.
[d]GI, glutaraldehyde-inactivated.
[e]FI, formalin-inactivated.
[f]Centre for Applied Microbiology and Research.

TABLE 2

IgG antibody responses to pertussis antigen and diphtheria and tetanus toxoids in adults and young children after immunization with placebo or acellular pertussis (AP), diphtheria-tetanus-pertussis (DTP), or multicomponent acellular DTP (ADTP) toxoids.

	Adults				Children			
	Before immunization		Postimmunization day 28		Before immunization		After immunization	
	Placebo	AP $CP_{10/5/5/3}$	Placebo	AP $CP_{10/5/5/3}$	DTP	ADTP $CP_{10/10/5/3}DT$	DTP	ADTP $CP_{10/10/5/3}DT$
Pertussis toxoid	16.45 (9.46–28.62)	22.78 (12.11–42.86)	16.56 (9.08–30.22)	415.87 (243.91–709.09)	43.71 (14.29–133.88)	15.45 (8.50–28.10)	221.32 (99.83–490.67)	306.55 (155.84–603.03)
Filamentous hemagglutinin	15.24 (10.28–22.60)	23.59 (15.59–35.69)	13.36 (7.71–23.16)	317.37 (243.05–141.41)	2.93 (1.81–4.73)	3.86 (3.03–4.93)	30.06 (11.82–76.46)	29.86 (16.51–53.99)
Agglutinogens	21.26 (12.14–37.23)	28.64 (12.20–67.21)	27.0 (15.37–47.78)	2048.00 (1025.62–4089.55)	26.72 (16.94–42.5)	29.24 (13.63–62.75)	315.2 (127.4–779.9)	1243.3 (594.8–2603.5)

TABLE 2-continued

IgG antibody responses to pertussis antigen and diphtheria and tetanus toxoids in adults and young children after immunization with placebo or acellular pertussis (AP), diphtheria-tetanus-pertussis (DTP), or multicomponent acellular DTP (ADTP) toxoids.

	Adults				Children			
	Before immunization		Postimmunization day 28		Before immunization		After immunization	
	Placebo	AP $CP_{10/5/5/3}$	Placebo	AP $CP_{10/5/5/3}$	DTP	ADTP $CP_{10/10/5/3}DT$	DTP	ADTP $CP_{10/10/5/3}DT$
Pertactin	7.89 (4.00–15.56)	11.47 (6.41–20.55)	7.46 (3.51–15.87)	855.13 (396.41–1844.67)	6.54 9.45 (2.79–15.33)	60.13 (5.50–16.23)	116.16 (24.59–147.04)	(57.87–233.19)
CHO cell neutralizing assay	12.30 (6.97–21.68)	21.11 (10.35–43.06)	10.78 (5.54–20.97)	604.67 (403.82–405.41)	27.47 (7.36–102.62)	9.71 (4.71–20.03)	270.60 (24.6–1100.8)	342.51 (146.6–800.2)
Diphtheria toxoid	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	8.75 (6.52–23.92)	9.65 (5.62–16.57)
Tetanus toxoid	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	4.11 (3.20–5.28)	6.32 (5.31–7.53)
No. studied	16	15	16	15	10	25	12	25

Data are expressed as geometric mean with 95% confidence intervals. For pertussis toxoid, filamentous hemagglutinin, agglutinogens, pertactin, and diphtheria and tetanus toxoids, antibody titers expressed as ELISA units/nL. For CHO cell neutralizing assay, values reflect reciprocal of highest dilution demonstrating 80% neutralization.

TABLE 3

Serologic Results of Acellular Pertussis Vaccines In Infants (2, 4 and 6 Months Old)

			Geometric Mean Titres								
Clinical Trial	Product	Study	Number of Participants	PT	FHA	69 kDa	Fimbrial agglutinogens	CHO Cell Neutralization	Agglutination	Tet	Dip
1	$CP_{10/5/5}DT$	U.S. NIAID	108	38	37	3	229	160	85	7.8	0.8
	$CP_{10/5/5/3}DT$	Multicentre	113	36	36	113	241	150	73	5.0	0.4
	Whole Cell (Mass.)	Comparative Study	95	20	51	101	70	80	42	—	—
	Whole Cell (Lederle)	(Cycle I)	312	67	3	64	193	270	84	—	—
2	$CP_{10/5/5/3}DT$	Phase II	315	87.1	50.2	29.9	239.8	29.6	—	1.5	0.3
	Whole Cell (CLL)	Canada	101	20	4.7	6.4	603.2	2.6	—	1.2	0.4
3	$CP_{10/5/5/3}DT$	Phase IIB	32	58.4	45.2	40.6	111.4	32.7	—	1.0	0.14
	$CP_{20/20/5/3}DT$	Canada	33	133.3	95.0	37.1	203.8	82.4		1.1	0.21
	Whole Cell (CLL)		30	10.4	8.9	6.8	393.9	4.0		1.8	0.31
4	$CP_{10/5/5/3}DT$	Phase IIC	42	105.1	82.5	71.1	358.6	66.9	307.0	2.0	0.33
	$CP_{20/20/5/3}DT$	Canada	250	101.6	163.9	87.6	220.6	68.7	219.2	1.8	0.38
5	$CP_{20/20/5/3}DT$	Montreal	58	212.7	83.4	106.3	601.9	109.6	—	1.9	0.53
	Whole Cell (CLL)	Feasibility Study	58	101.4	11.7	16.8	906.9	6.0		1.1	0.27
6	$CP_{10/5/5}DT$	U.S. NIAID	80	42	34	50	310	196	185		
	$CP_{20/20/5/3}DT$	Comparative Study	80	39	87	43	184	254	137	—	—
	Whole Cell (CLI)	(Cycle II)	80	2	3	9	33	54	167		
	Whole Cell (Lederle)		80	18	2	16	129	137	86		

CLI - Connaught Laboratories Incorporated, Swiftwater, Pennsylvania.
Mass - Massachusetts Public Laboratories.
CLL - Connaught Laboratories Limited, Willowdale, Ontario.
Lederle - Lederle Laboratories Inc.

What we claim is:

1. A process for preparing an agglutinogen preparation comprising fimbrial agglutinogens 2 (Agg 2) and fimbrial agglutinogen 3 (Agg 3) free from agglutinogen 1 from a Bordetella strain, comprising the steps of:

(a) providing a cell paste of the Bordetella strain;

(b) selectively extracting fimbrial agglutinogens 2 and 3 from the cell paste by dispersing the cell paste in a buffer comprising about 1M to about 6M urea to produce a first supernatant containing said agglutinogens 2 and 3 and a first residual precipitate;

(c) separating the first supernatant from the first residual precipitate;

(d) incubating the first supernatant at a temperature of about75° C. to about 85° C. and for a time of about 10 minutes to about 60 minutes to produce a clarified supernatant containing fimbrial agglutinogens 2 and 3 and a second precipitate containing non-fimbrial agglutinogen contaminants;

(e) concentrating the clarified supernatant to produce a crude fimbrial agglutinogen solution by precipitating fimbrial agglutinogens 2 and 3 from the clarified supernatant by the addition of a polyethylene glycol to the clarified supernatant, separating the precipitated fimbrial agglutinogen 2 and 3 from the resulting supernatant and solubilizing the separated fimbrial agglutinogens 2 and 3; and

(f) purifying fimbrial agglutinogens 2 and 3 from the crude fimbrial agglutinogen solution to produce the fimbrial agglutinogen preparation comprising fimbrial agglutinogens 2 and 3.

2. The process of claim **1** wherein the temperature is about 80° C.

3. The process of claim **1** wherein the time is about 30 minutes.

4. The process of claim **1** wherein the first supernatant is concentrated prior to the incubation step (d).

5. The process of claim **1** wherein said precipitation is effected by adding polyethylene glycol of molecular weight about 8000 to the clarified supernatant to a concentration of about 3% to about 5 wt. % to effect precipitation of said agglutinogens from the clarified supernatant.

6. The process of claim **5** wherein the concentration of polyethylene glycol is about 4.3 to about 4.7wt%.

7. The process of claim **1** wherein the agglutinogens are purified from the crude fimbrial agglutinogen solution by column chromatography.

8. The process of claim **7** wherein said column chromatography includes SEPHADEX 6B and/or PEI silica column chromatography.

9. The process of claim **7** wherein said purification step includes sterilization of run through from said column chromatography purification to provide a sterile fimbrial agglutinogen preparation.

10. The process of claim **9** wherein said sterile fimbrial agglutinogen preparation is absorbed onto a mineral salt adjuvant.

11. The process of claim **10** wherein said mineral salt adjuvant is alum.

12. The process of claim **1** wherein the Bordetella strain is a strain of *Bordetella pertussis*.

* * * * *

EXHIBIT G

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,877,298
DATED : March 2, 1999
INVENTOR(S) : Raafat E.F. Fahim et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item [73] Assignee should be inserted --Connaught Laboratories Limited--.

Signed and Sealed this

Twenty-sixth Day of October, 1999

Attest:

Q. TODD DICKINSON

Attesting Officer

Acting Commissioner of Patents and Trademarks

EXHIBIT H

United States Patent and Trademark Office

Patent Maintenance Fees			08/14/2008 11:35 AM EDT
Patent Number:	5877298	**Application Number:**	08433646
Issue Date:	03/02/1999	**Filing Date:**	05/04/1995
Window Opens:	03/02/2010	**Surcharge Date:**	09/03/2010
Window Closes:	03/02/2011	**Payment Year:**	
Entity Status:	LARGE		
Customer Number:	1267		
Street Address:	SIM & MC BURNEY 330 UNIVERSITY AVENUE		
City:	TORONTO		
State:	ON		
Zip Code:	M5G 1R7		
Phone Number:	CA +1 4165951155 Call		
Currently there are no fees due.			

EXHIBIT I

Clinical Trial	Start Date	End Date
494-01 Stage I	December 29, 1999	April 05, 2001
494-03 Stage I	July 10, 2000	August 6, 2001
5A9908	August 15, 2000	October 21, 2001
494-01 Stage II	February 2, 2001	April 23, 2002
P3T06 Stage I	May 4, 2001	October 25, 2002
494-03 Stage II	May 8, 2001	December 26, 2002
P3T06 Stage II	June 12, 2002	January 21, 2004
M5A07 Stage I	October 30, 2003	January 19, 2005
M5A07 Stage II	August 30, 2004	March 30, 2006
M5A10 Stage I	November 10, 2005	April 16, 2007